	L #	Hits	Search Text	DBs	Time Stamp
1	L1	436	ikk$2	USPAT; US-PGPUB	2003/11/04 09:50
2	L2	584058	complex	USPAT; US-PGPUB	2003/11/04 09:50
3	L3	120	1 same 2	USPAT; US-PGPUB	2003/11/04 09:51
4	L4	25	spa-1	USPAT; US-PGPUB	2003/11/04 14:41
5	L5	3	1 and 4	USPAT; US-PGPUB	2003/11/04 14:41
6	L6	6	4 same 2	USPAT; US-PGPUB	2003/11/04 14:43
7	L7	2341	nfkb or (nf adj kappa adj b) or (nf adj kb)	USPAT; US-PGPUB	2003/11/04 14:45
8	L8	2	6 and 7	USPAT; US-PGPUB	2003/11/04 14:46
9	L9	7	4 and 7	USPAT; US-PGPUB	2003/11/04 14:46

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	436	ikk$2	USPAT; US-PGPUB	2003/11/04 09:50
2	L2	584058	complex	USPAT; US-PGPUB	2003/11/04 09:50
3	L3	120	1 same 2	USPAT; US-PGPUB	2003/11/04 09:51

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030203926 A1

TITLE: Anilinopyrimidine derivatives as IKK inhibitors and compositions and methods related thereto

PUBLICATION-DATE: October 30, 2003

INVENTOR-INFORMATION:

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DATE FILED: December 4, 2001

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US-CL-CURRENT: 514/275, 514/252.14 , 544/295 , 544/330 , 544/331

ABSTRACT:

Compounds having activity as inhibitors of IKK are disclosed, particularly IKK-2. The compounds of this invention are anilinopyrimidine derivatives having the following structure: 1
wherein R.sub.1 and R.sub.6 are as defined herein. Such compounds have utility in the treatment of a wide range of conditions that are responsive to IKK inhibition. Thus, methods of treating such conditions are also disclosed, as are pharmaceutical compositions containing one or more compounds of the above compounds.

[0001] This application claims the benefit of U.S. Provisional Application No. 60/251,816, filed Dec. 6, 2000, incorporated by reference herein in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0004] The I.kappa.B kinases (**IKKs**), are key regulatory signaling molecules coordinating the activation of NF-.kappa.B. **IKK-1 and IKK-2** are structurally unique kinases containing an N-terminal kinase domain with a dual serine activation loop, a leucine zipper domain, and a C-terminal helix-loop-helix domain and serine cluster. **IKK** enzymes show relatively low sequence homologies with other kinases, and early profiles with known kinase inhibitors have not identified compounds with striking potency. Kinetic analysis shows that **IKK-2** binds to and phosphorylates I.kappa.B.alpha., I.kappa.B.alpha., and IKB.epsilon. with high and relatively equal affinities (Heilker et.al. 1999). Recombinant **IKK-2** phosphorylates I.kappa.B.alpha. peptide 26-42 with near equal affinity to full length I.kappa.B.alpha., however the native **IKK** enzyme **complex** phosphorylates full length I.kappa.B.alpha. 25,000 fold more efficiently, suggesting important regulatory sequences in the C-terminal region of I.kappa.B.alpha., or additional regulatory proteins in the **IKK** enzyme **complex** that accelerate the rate of catalysis (Burke et al., Journal of Biological Chemistry 274:36146-36152, 1999). Phosphorylation of I.kappa.B.alpha. occurs via a random sequential kinetic mechanism, meaning either ATP or I.kappa.B.alpha. may bind first to **IKK-2**, t that both must be bound before phosphorylation of I.kappa.B.alpha. can take place (Peet and Li, Journal of Biological Chemistry 274:32655-32661, 1999). **IKK-2** binds ATP with uniquely high affinity (Ki=130 nM) compared to other serine-threonine kinases such as p38 and JNK perhaps indicating a unique ATP binding pocket that reflects the relatively poor activity to many broad specificity kinase inhibitors when tested against **IKK-2**. To date, no crystal structure of **IKK-2** has been reported. However homology modeling has identified 3 structural domains including an N-terminal kinase domain with an activation loop, a leucine zipper domain that likely mediates the formation of **IKK-1 and IKK-2** homo/heterodimers, and a C-terminal helix-loop-helix with serine rich tail. Activation of **IKK-2** is critically dependent upon phosphorylation of serine 177 and 181 in the activation or T loop. Alanine mutations abolish activity, while glutamate mutations result in a constitutively active enzyme (Mercurio et al. Science 278:860-866, 1997; Delhase et al., Science 284:30 313, 1999).

Summary of Invention Paragraph - BSTX (6):

[0005] **IKK-1 and IKK-2** occur both as heterodimers and **IKK-2** homodimers, and are associated with a 700-900 kDa cytoplasmic enzyme **complex** called the "**IKK** Signalsome" (Mercurio et al., Science 278:860-866, 1997). Another component, **IKKAP**-1 or NEMO/IKK.gamma. has no apparent catalytic function but will associate directly with **IKK-2** and is necessary for full activation of NF-.kappa.B (Mercurio et al., Mol Cell Biol 19:1526-1538, 1999). Many immune and inflammatory mediators including TNF.alpha., lipopolysaccharide (LPS), IL-1, anti-CD28, CD40L, FasL, viral infection, and oxidative stress have been shown to lead to NF-.kappa.B activation. Although the receptor complexes that transduce these diverse stimuli appear very different in their protein components, it is understood that each of these stimulation events leads to activation of the **IKKs** and NF-.kappa.B.

Summary of Invention Paragraph - BSTX (7):

[0006] The **IKK complex** appears to be the central integrator of diverse inflammatory signals leading to the phosphorylation of I .kappa.B. **IKKs** are

activated at dual serine residues by upstream kinases including NF-.kappa.B inducing kinase, NIK (Malinin et al., Nature 385:540-544, 1997), and MEKK-1 (Yujiri et al., Science 282:1911-1914, 1998). The differential activities of NIK and MEKK-1 remain unclear although initial data indicates these kinases may preferentially activate **IKK-1 and IKK-2,** respectively. Activated **IKK** phosphorylates a cytoplasmic inhibitor protein, I.kappa.B which binds NF-.kappa.B, thereby masking a nuclear localization signal present in Rel proteins (Cramer et al., Structure 7:R1-R6, 1999). **IKK** phosphorylation of I.kappa.B on serines 32 and 36 forms a structural motif recognized by the E3 ligase, .beta.TRcP (Yaron et al., Nature 396:590-594, 1998). Docking of .beta.TRcP results in the formation of a ligase **complex** which polyubiquitinates I.kappa.B thus targeting it for degradation by the 26S proteosome. Free NF-.kappa.B is then identified by nuclear transport proteins which translocate it to the nucleus where it can associate with sequence specific regulatory elements on gene promoters.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030199462 A1

TITLE: Methods and compositions for regulating cellular signaling

PUBLICATION-DATE: October 23, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 128174

DATE FILED: April 23, 2002

US-CL-CURRENT: 514/44, 435/15 , 435/194 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The present invention related to methods and compositions for modulating cellular signaling. In particular, the present invention relates to PKK and RICK3 proteins. The present invention further relates the to use of PKK and RICK3 proteins in modulating NF-.kappa.B signaling. The present invention thus provides novel targets for drug screening and therapeutics.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (6):

[0023] FIG. 5 demonstrates that PKK Acts through the **IKK complex** and independently of Bcl10 to activate NF-.kappa.B. FIG. 5(A) shows that PKK-induced NF-.kappa.B activation is inhibited by dominant negative forms of IKK.alpha. and IKK.beta. but not by those of IKK.gamma., Bimp1, nor MyD88. Induction of NF-.kappa.B activation was determined in triplicate cultures of HEK293T cells transfected with 1.6 ng of pcDNA3-Myc-PKK, or stimulated with 50 ng/ml PMA and 0.7 .mu.g/ml of A23187, 10 ng/ml IL-1.beta. or 10 ng/ml TNF.alpha. for 4 hrs in the presence of pBVIx-Luc and pEF-BOS-.beta.-gal. Results are presented as a percent of values obtained with PKK and control plasmid. In the experiment shown, PKK, PMA/Ca.sup.2+-ionophore, IL-1.beta. and TNF.alpha. induced 55.+-.3, 196.+-.15, 423.+-.22 and 183.+-.55 fold activation of NF-.kappa.B, respectively. Values represent mean of normalized values .+-.SD of triplicate cultures. FIG. 5(B) shows that PKK-mediated NF-.kappa.B activation requires IKK.alpha. and IKK.beta.. Induction of

NF-.kappa.B activation was determined in wt, IKK.alpha..sup.-/-, IKK.beta..sup.-/- and IKK.alpha..sup.-/-/IKK.beta..sup.-/- mouse embryonic fibroblasts transfected with 100 ng of pcDNA3-Flag-PKK, pcDNA3-Nod1-Flag and pcDNA-IKK.beta.-Myc in the presence of pBVIx-Luc and pEF-BOS-.beta.-gal. FIG. 5(C) shows induction of NF-.kappa.B in parental Rat-1 and IKK.gamma.-deficient 5R cells. Induction of NF-.kappa.B activation was determined in wt, IKK.alpha..sup.-/-, IKK.beta..sup.-/- and IKK.alpha..sup.-/-/IKK.beta..sup.-/- mouse embryonic fibroblasts transfected with 100 ng of pcDNA3-Flag-PKK, pcDNA3-Nod1-Flag and pcDNA-IKK.beta.-Myc in the presence of pBVIx-Luc and pEF-BOS-.beta.-gal. FIG. 5(D) shows PKK-mediated activation of NF-.kappa.B in the absence of Bcl10. Bcl10.sup.+/- and Bcl10.sup.-/- mouse embryonic fibroblasts were transfected with 900 ng of the indicated expression plasmid: pcDNA3-Flag-PKK, pcDNA3-Nod1-HA or pcDNA3-Bimp1-Flag.

Detail Description Paragraph - DETX (107):

[0162] Protein kinase C-associated kinase (PKK/DIK) is a recently described kinase of unknown function that was identified on the basis of its specific interaction with PKC.beta.. PKK/DIK contains N-terminal kinase and C-terminal ankyrin repeats domains linked to an intermediate region. Experiments conducted during the course of development of the present invention revealed that the kinase domain of PKK/DIK is highly homologous to that of two mediators of nuclear factor-.kappa.B (NF-.kappa.B) activation, RICK and RIP, but these related kinases have different C-terminal domains for binding to upstream factors. Expression of PKK, like RICK and RIP, was found to induce NF-.kappa.B activation. Mutational analysis revealed that the kinase domain of PKK is essential for NF-.kappa.B activation whereas replacement of serine residues in the putative activation loop did not affect the ability of PKK to activate NF-.kappa.B. A catalytic inactive PKK mutant inhibited NF-.kappa.B activation induced by phorphol ester and Ca.sup.2+-ionophore but it did not block that mediated by tumor necrosis factor .alpha., interleukin-1.beta. or Nod1. Inhibition of NF-.kappa.B activation by dominant negative PKK was reverted by co-expression of PKC.beta.I, suggesting a functional association between PKK and PKC.beta.I. PKK-mediated NF-.kappa.B activation required IKK.alpha. and IKK.beta. but not IKK.gamma., the regulatory subunit of the **IKK complex**. Moreover, NF-.kappa.B activation induced by PKK was not inhibited by dominant negative Bimp1 and proceeded in the absence of Bcl10, two components of a recently described PKC signaling pathway. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that these results suggest that PKK is a member of the RICK/RIP family of kinases which is involved in a PKC-activated NF-.kappa.B signaling pathway that is independent of Bcl10 and IKK.gamma..

Detail Description Paragraph - DETX (303):

[0352] NF-.kappa.B activation by RICK and RIP is mediated by the **IKK complex**, a universal regulator, which phosphorylates I.kappa.B.alpha. resulting in degradation of I.kappa.B.alpha. and nuclear translocation of NF-.kappa.B (Karin and Ben-Neriah, Annu. Rev. Immunol. 18:621 [2000]; Inohara et al., [2000], supra). To determine whether NF-.kappa.B activation by PKK is also dependent on **IKKs**, PKK was co-expressed with the catalytic inactive forms of IKK.alpha. and IKK.beta.. As it was found with its related RICK and

RIP kinases (Inohara et al., [2000], supra), NF-.kappa.B activation induced by PKK as well as that induced by PMA/Ca.sup.2+-ionophore, IL-1.beta. and TNF.alpha., was inhibited by catalytic inactive IKK.alpha. and IKK.beta. (FIG. 5A). In control experiments, PKK-mediated NF-.kappa.B activation was not affected by dominant negative forms of Bimp1 or MyD88 (FIG. 5A). The ability of PKK to activate NF-.kappa.B was also determined in mouse embryonic fibroblasts lacking IKK.alpha. and IKK.beta.. Whereas NF-.kappa.B was activated in wt fibroblasts, PKK failed to induce NF-.kappa.B activation in cells lacking both IKK.alpha. and IKK.beta., and was greatly impaired in fibroblasts lacking IKK.beta. (FIG. 5B). These results suggest that NF-.kappa.B activation induced by PKK requires catalytic proteins of **IKKs**.

Detail Description Paragraph - DETX (304):

[0353] It was next tested if NF-.kappa.B activation by PKK requires IKK.gamma., a regulatory component of **IKK complex** (Mercurio et al., Mol. Cell Biol. 19:1526 [1999]; Rothwarf et al., Nature 395:297 [1998]; Li et al., Proc. Natl. Acad. Sci. USA. 96:1042 [1999]). PKK was co-expressed with a truncated mutant of IKK.gamma. (residues 134-419), which inhibits NF-.kappa.B activation induced by RIP and RICK (Inohara et al., [2000], supra). Co-expression of the IKK.gamma. mutant did not inhibit PKK-mediated NF-.kappa.B activation (FIG. 5A). To verify the latter result, the ability of PKK to activate NF-.kappa.B in parental Rat1 fibroblasts and IKK.gamma.-deficient 5R cells, a Rat1 derivative cell line that is defective in IKK.gamma. (Yamaoka et al., Cell 93:1231 [1998]) was tested. Expression of PKK induced NF-.kappa.B activity not only in parental Rat1 cells but also in 5R cells (FIG. 5C). As a control, stimulation with TNF.alpha., IL-1.beta., LPS, or expression of Nod1, which require IKK.gamma., induced NF-.kappa.B activation in parental Rat1 but not in 5R cells (FIG. 5C). FIG. 3 shows that the IM region of PKK is not essential for NF-.kappa.B activation, in contrast, the same region of RIP and RICK is essential for NF-.kappa.B activation and mediates the interaction with IKK.gamma. (Inohara et al., [2000], surpra, Li et al., supra).

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TITLE: Inflammation modulators

PUBLICATION-DATE: September 25, 2003

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DATE FILED: December 4, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60337460 20011205 US

US-CL-CURRENT: 514/266.23, 514/266.21 , 514/314 , 544/284 , 546/167

ABSTRACT:

Compounds, compositions and methods that are useful in the treatment of inflammatory, immunoregulatory, metabolic and cell proliferative conditions or diseases are provided herein. In particular, the invention provides compounds which modulate the expression and/or function of proteins involved in inflammation, metabolism and cell proliferation. The subject compounds contain quinoline or quinazoline rings.

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of provisional application Ser. No. 60/337,460, filed Dec. 5, 2001, the entire contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0005] In its inactive state, the NF-.kappa.B heterodimer is held in the cytoplasm by association with inhibitory IkB proteins. Recently, the three-dimensional structure of a NF-.kappa.B/I.kappa.B ternary **complex** has been solved (Huxford et al. Cell, 95, 759 (1998); Jacobs et al. Cell, 95, 749 (1998)). When cells are treated with the appropriate stimuli, such as IL-1 or TNF, intracellular signal transduction pathways are activated that lead to the eventual phosphorylation of IkB proteins on two specific residues (serines 32 and 36 in IkB.alpha., serines 19 and 23 in IkB .beta.). Mutation of one or both serine residues renders IkB resistant to cytokine-induced phosphorylation. This signal-induced phosphorylation targets IkB for ubiquitination and proteosome-mediated degradation, allowing nuclear translocation of NF-.kappa.B (Thanos and Maniatis, Cell, 80, 529 (1995)). The only regulated step in the IkB degradation pathway is the phosphorylation of IWB by IkB kinases (**IKK**) (Yaron et al. EMBO J. 16, 6486 (1997)).

Summary of Invention Paragraph - BSTX (6):

[0006] Several intermediate steps in the TNF- and IL-1-activated signaling pathways that result in IkB phosphorylation have been elucidated in recent years. Both pathways appear to merge at the level of the protein kinase NIK (NF-.kappa.B-inducing kinase) (Malinin et al. Nature, 385, 540 (1997); Song et al. Proc. Natl. Acad. Sci. USA, 94, 9792 (1997)). Similarly, the protein kinases MEKK1 and MLK3 have been implicated in the induction of **IKK** activity (Lee et al. Proc. Natl. Acad. Sci. USA. 95, 9319 (1998); Hehner et al. Mol. Cell. Biol. 20, 2556 (2000)). While the specific details remain somewhat unclear regarding how these or other intermediate proteins may interact with and/or stimulate **IKK** activity in cells, significant progress has been made in elucidating the enzymes responsible for IkB phosphorylation. Two **IKK** enzymes, generally referred to as IKK.alpha. and IKK.beta. (Woronicz et al. Science, 278, 866 (1997); Zandi et al. Cell, 91, 243 (1997)) or **IKK-1 and IKK-2** (Mercurio et al. Science, 278, 860 (1997)) have been discovered. Both forms of **IKK** can exist as homodimers and as IKK.alpha./IKK.beta. heterodimers. Another recently discovered component of the IkB kinase **complex** is a regulatory protein, known as **IKK**-gamma or NEMO (NF-.kappa.B-Essential Modulator) (Rothwarf et al. Nature, 395, 297 (1998)). NEMO does not contain a catalytic domain, and thus it appears to have no direct kinase activity and it probably serves a regulatory function. Existing data suggest that the predominant form of **IKK** in cells is an IKK.alpha./IKK.beta. heterodimer associated with either a dimer or a trimer of NEMO (Rothwarf et al. Nature 395, 297 (1998)).

PGPUB-DOCUMENT-NUMBER: 20030176373

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030176373 A1

TITLE: Agents that modulate DNA-PK activity and methods of use thereof

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

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RELATED-US-APPL-DATA:

non-provisional-of-provisional 60202274 20000505 US

non-provisional-of-provisional 60262321 20010117 US

US-CL-CURRENT: 514/44, 435/455 , 435/6

ABSTRACT:

The present invention provides methods for modulating cell death in a eukaryotic cell, and methods for reducing DNA damage in a eukaryotic cell. The methods generally comprise modulating a biological activity of DNA-PK in a cell. The invention further provides methods of treating a condition related to cell death in an individual. The invention further provides methods of identifying agents which modulate a biological activity of DNA-PK, as well as agents identified by the methods. Methods of modulating an immune response using an identified agent are also provided.

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application serial No. 60/202,274, filed May 5, 2000, and U.S. Provisional Application serial No. 60/262,321, filed Jan. 17, 2001, both of which are incorporated by reference herein in their entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (181):

[0203] Kinase assays and immunoblotting were performed according to Li et al. ((1999) J. Exp. Med. 189:1839-1845). Briefly, BMDM were treated with ISS-ODN (5 .mu.g/ml), M-ODN (5 .mu.g/ml) on ps and po backbones as indicated, LPS-free bacterial DNA or methylated bacterial DNA (5 .mu.g/ml), LPS-free calf thymus DNA (5 .mu.g/ml), LPS (10 .mu.g/ml) or TNF.alpha. (10 ng/ml) for the indicated time periods. Cell lysates were prepared and normalized by immunoblotting (IB) with anti-IKK.alpha. polyclonal antibodies (Santa Cruz, Santa Cruz Biotech Inc., Calif.), anti-IKK.beta. polyclonal antbodies (Santa Cruz) or anti-DNA-PKcs monoclonal antibodies (NeoMarker, Calif.). I.kappa.B kinase (**IKK) complex** or DNA-PK **complex** from 100 .mu.g of the lysates were immunoprecipitated by anti-IKK.alpha. or by anti-DNA-PKcs antibodies. The kinase activities (KA) were determined by a kinase assay using the N-terminus of I.kappa.B.alpha. (for **IKK**) or the N-terminus of p53 (for DNA-PK) as a substrate as previously described. Wang et al. (1992) Proc. Natl. Acad. Sci. USA 89:4231-4235; Li et al.(1999), supra; and Hammarsten et al. (2000) J. Biol. Chem. 275:1541-1545.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175762 A1

TITLE: Modulators on Nod2 signaling

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 314506

DATE FILED: December 9, 2002

RELATED-US-APPL-DATA:

child 10314506 A1 20021209

parent continuation-in-part-of 10014269 20011026 US PENDING

non-provisional-of-provisional 60244289 20001030 US

US-CL-CURRENT: 435/6, 435/7.21 , 514/8

ABSTRACT:

The present invention relates to intracellular signaling molecules, in particular the Nod2 protein and nucleic acids encoding the Nod2 protein. The present invention provides methods of identifying modulators of Nod2 signaling. In particular, the present invention additionally provides methods of screening immune modulators such as adjuvants using Nod2. The present invention further provides methods of altering Nod2 signaling.

[0001] This application is a continuation in part of U.S. patent application Ser. No. 10/014,269, filed Oct. 26, 2001, which claims priority to U.S. provisional patent application serial No. 60/244,289, filed Oct. 30, 2000, each of which is herein incorporated by reference in its entirety.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (6):

[0022] FIG. 5 shows that Nod2 Acts through the **IKK compl x** to activate NF-KB. FIG. 5A shows inhibition of Nod2 and TNF.alpha.-induced NF-KB

activation by dominant negative mutant proteins of the NF-.kappa.B pathway. Induction of NF-.kappa.B activation was determined in triplicate cultures of HEK293T cells transfected with 30 ng of Nod2 plasmid (open bars) or stimulated with 10 ng/ml of TNF.alpha. for 4 h (closed bars) and 70 ng of I-.kappa.B.alpha. S32A/S36A, IKK.alpha. K44A, IKK.beta. K44A, RICK (406-540) or RIP (558-671) expression plasmid in the presence of pBVIx-Luc and pEF-BOS-.beta.-gal. Results are presented as a percent of values obtained with Nod2 and control plasmid. In the experiment shown, Nod2 and TNF.alpha. induced 58.+-.8-fold and 14.+-.1-fold activation of NF-.kappa.B, respectively. Values represent mean .+-.SD of triplicate cultures. FIG. 5B shows induction of NF-.kappa.B in parental Rat-1 and derivative 5R cells. Induction of NF-.kappa.B activation was determined from triplicate cultures of l.times.10.sup.5 HEK293T cells co-transfected with the indicated plasmids and pBVIx-Luc in the presence of control plasmid pEF-BOS-.beta.-gal. Values represent mean .+-.SD of triplicate cultures.

Detail Description Paragraph - DETX (5):

[0049] Nod2 is the first molecule known to contain two CARDS. The molecular basis underlying the requirement of both CARDs of Nod2 for RICK binding remains unclear. The present invention is not limited to any particular mechanism of action. Indeed, an understanding of the mechanism of action is not necessary to practice the present invention. Nevertheless, it is contemplated that the presence of both CARDs may enhance the affinity for the CARD of RICK. Another possibility is that upon an initial interaction involving a CARD of Nod2 and the CARD of RICK, Nod2 may undergo a conformational change that allows the second CARD to associate with high affinity to RICK. The intermediate region of RICK associates with IKK.gamma. (Inohara et al., [2000], supra), providing a direct link between Nod1/Nod2 and the **IKK complex**. Consistent with this model, NF-.kappa.B activation induced by Nod2 as well as that induced by Nod l required IKK.gamma. and was inhibited by dominant negative forms of IKK.gamma., IKK.alpha. and IKK.beta.. The functional role for the LRRs of Nod1 and Nod2 remains unclear. The LRR is a repeated protein-protein interaction module that is presumably involved in the activation of Nod1 and Nod2 by upstream signals. In the case of plant NBD/LRR-containing R proteins, their LRRs appear to be important for the recognition of pathogen components and their N-terminal domains appear to mediate a signaling cascade that regulates gene expression (Parniske et al., supra, Dixon et al., supra). Because both Nod1 and Nod2 activate NF-.kappa.B, their LRRs may act to recognize a different set of intracellular stimuli that mediate Nod1 and Nod2 oligomerization and association with RICK. Nod2 is expressed in monocytes, dendritic cells, and paneth cells in the gut (Gutierrez et al., J Biol Chem 277(44):41701-5 [2002]). Because Nod2 is expressed in monocytes, Nod2 might serve as an intracellular receptor that transduces signals in the monocyte/macrophage that lead to activation of NF-.kappa.B and transcription of regulatory genes.

Detail Description Paragraph - DETX (343):

[0379] This example demonstrates that NF-.kappa.B activation induced by Nod2 requires IKK.gamma. and is inhibited by dominant negative forms of **IKKs** and RICK. A main pathway of NF-.kappa.B activation is mediated by I.kappa.B kinases (**IKKS**) resulting in I.kappa.B phosporylation and release of cytoplasmic

NF-.kappa.B (Karin, J. Biol. Chem. 274: 27339-27342 [1999]). To determine whether Nod2 activates an **IKK**-dependent pathway, Nod2 was co-expressed with mutant forms of IKK.alpha., IKK.beta., and I.kappa.B that have been shown to act as dominant inhibitors of their corresponding endogenous counterparts and/or the **IKK complex** (Karin, supra). In addition, a truncated mutant of IKK.gamma./Nemo (residues 134-419) was used that is defective in IKK.alpha. and IKK.beta. binding and acts as an inhibitor of NF-.kappa.B activation induced by RIP and RICK (Inohara et al., [2000], supra). The NF-.kappa.B activity induced by Nod2 as well as that induced by TNF.alpha. stimulation were greatly inhibited by mutant IKK.alpha., IKK.beta., IKK.gamma., and I.kappa.B.alpha. (FIG. 5A). Because RICK has been shown to serve as a downstream target of Nod1 (Bertin et al., supra, Inohara et al., [1999] supra, Inohara et al., [2000], supra), a truncated form of RICK containing its CARD (residues 406-540) that acts as a dominant inhibitor of Nod1 activity (Bertin et al., supra) was used to test whether NF-.kappa.B activation induced by Nod2 is similarly inhibited by this RICK mutant. NF-.kappa.B activation induced by Nod2 was inhibited by mutant RICK but not by a mutant form of RIP that expresses its death effector domain (FIG. 5A). The inhibition by the CARD of RICK was specific in that it did not interfere with ability of TNF.alpha. to induce NF-.kappa.B, an activity that was inhibited by the RIP mutant (FIG. 5A). To verify that Nod2 acts upstream of the **IKK complex** to activate NF-.kappa.B, we tested the ability of Nod2 to activate NF-.kappa.B in parental Rat1 fibroblasts and 5R cells, a Rat1 derivative cell line that is defective in IKK.gamma., an essential subunit of the **IKKs** (Yamaoka et al., supra). Nod2, as well as Nod1 and TNFoc, induced NF-KB activity in parental Rat1 cells but not in IKK.gamma.-deficient 5R cells (FIG. 5B). As a control, expression of IKK.beta., which functions downstream of IKK.gamma., induced NF-.kappa.B activation in both Rat1 and 5R cell lines (FIG. 5B). These results indicate that Nod2 acts through IKK.gamma./**IKK**/IKK.beta. to activate NF-.kappa.B.

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TITLE: Methods and compositions relating to modulation of A20

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INVENTOR-INFORMATION:

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US-CL-CURRENT: 514/1, 435/7.23

ABSTRACT:

The invention provides compositions and methods for treating diseases characterized by aberrant programmed cell death and/or inflammation, comprising mediating A20 function in the subject. Such diseases include Crohn's disease, inflammatory bowel disease, a disease associated with ischemic injury, a toxin-induced liver disease and cancer. The invention further provides methods and compositions for assays for modulators of A20.

[0001] This application claims the priority of U.S. Provisional Patent Application Serial No. 60/285,427, filed Apr. 19, 2001, the entire disclosure of which is specifically incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (6):

[0006] TNF is elaborated from macrophages as well as multiple other cell types, in response to stimuli such as IL-1, LPS and TNF itself. TNF binds to two TNF receptors, TNFR1 and TNFR2, stimulating several signaling pathways, including NF-.kappa.B, JNK and caspase mediated programmed cell death (PCD) pathways (Chan et al., 2000). Binding of TNF to TNFR1 and TNFR2 activates several proteins, including the RIP kinase, which in turn leads to activation of the inhibitor of kinase kinase (**IKK) complex,** comprised of IKK.alpha.,

IKK.beta. and IKK.gamma.. **IKK** phosphorylates I.kappa.B.alpha., which is then degraded by proteasomes and releases active NF-.kappa.B to traverse to the nucleus and mediate transcription of NF-.kappa.B target genes (Karin and Ben-Neriah, 2000). Many of these genes are pro-inflammatory genes, such as TNF, IFN-.gamma., IL-1, IL-6, IL-8, IL-12, MCP-1, P-selectin, E-selectin, and iNOS. The increased expression of these proteins facilitates inflammatory reactions by supporting the activation and differentiation of immune cells, recruiting additional immune cells to sites of inflammation, facilitating the passage of immune cells from the circulation across endothelial cells into inflamed tissues, and stimulating the elaboration of additional proinflammatory factors. Thus, TNF mediates pro-inflammatory cascades via NF-.kappa.B activation. The importance of NF-.kappa.B activation to human disease is highlighted by observations that NF-.kappa.B dependent cytokines are elevated in human inflammatory bowel disease patients (Neurath et al., 1998). Thus, persistent TNF and NF-.kappa.B activity are associated with conditions such as bowel inflammation in both experimental models and human disease.

Detail Description Paragraph - DETX (201):

[0223] A20 inhibits NF-.kappa.B activation (Cooper t al., 1996), and dysregulated NF-.kappa.B activity leads to inflammation and premature death in I.kappa.B.alpha..sup.-/- mice (Beg et al., 1995). Moreover, the perturbed skin differentiation seen in A20.sup.-/- mice resembles the skin of I.kappa.B.alpha..sup.-/- mice (Beg et al., 1996). Thus, the pathogenesis of A20.sup.-/- mice may be due in part to dysregulated NF-.kappa.B activity. Repeated TNF treatment of normal MEFs caused I.kappa.B.alpha. degradation and NF-.kappa.B binding to DNA, followed by down-regulation of NF-.kappa.B binding and re-accumulation of I.kappa.B.alpha. protein by 60 min (FIG. 18 and FIG. 20). In contrast, NF-.kappa.B binding to DNA persisted and I.kappa.B.alpha. protein was not detected in A20.sup.-/- MEFs from 60-180 min of TNF treatment (FIG. 18 and FIG. 20). I.kappa.B.alpha. mRNA levels, transcriptionally enhanced by NF-.kappa.B (Sun et al., 1993), increased in response to TNF in both A20.sup.+/+ and A20.sup.-/- MEFs, indicating that the failure of A20.sup.-/- MEFs to re-accumulate I.kappa.B.alpha. protein was not due to a failure to express I.kappa.B.alpha. mRNA (FIG. 21). Addition of the proteasome inhibitor MG-132 to MEFs 15 min after TNF treatment caused A20.sup.-/- MEFs to regain normal levels of I.kappa.B.alpha. protein (FIG. 22A top panels), suggesting that the lack of I.kappa.B.alpha. protein re-accumulation in TNF treated A20.sup.-/- MEFs was due to rapid degradation of newly synthesized I.kappa.B.alpha. protein, rather than the failure of these cells to translate I.kappa.B.alpha. mRNA. I.kappa.B.alpha. protein which re-accumulated in MG-132 treated A20.sup.-/- but not A20.sup.+/+ MEFs was phosphorylated (FIG. 22A bottom panels), suggesting that persistent **IKK** (a multimeric **complex** comprised of IKK.alpha., IKK.beta., and IKK.gamma.) activity caused rapid phosphorylation of newly synthesized I.kappa.B.alpha. protein in TNF treated A20.sup.-/- MEFs. Direct measurement of **IKK** activity in lysates from TNF treated MEFs confirmed this suggestion (FIG. 22B). Therefore, synthesis of I.kappa.B.alpha. mRNA and I.kappa.B.alpha. protein is insufficient to terminate NF-.kappa.B signals in the absence of A20.

Detail Description Paragraph - DETX (220):

[0234] This finding suggests that A20 may either regulate translation of

I.kappa.B.alpha. mRNA, or prevent degradation of newly synthesized I.kappa.B.alpha. protein. A20 might perform the latter function by inhibiting the phosphorylation activity of the enzyme **complex** inhibitor of kinase kinase (**IKK**), or a more proximate step between TNFR and **IKK**. To distinguish between these potential functions for A20, TNF treated MEFs were treated with the proteosome inhibitor MG-132 fifteen minutes after TNF treatment. This dual treatment led to the reaccumulation of I.kappa.B.alpha. protein in A20.sup.-/- MEFs, suggesting that I.kappa.B.alpha. protein is synthesized normally in the absence of A20, but is rapidly degraded by proteosome dependent pathways (FIG. 22A). As the rapid degradation of I.kappa.B.alpha. protein could be due to rapid phosphorylation of I.kappa.B.alpha. by **IKK, IKK** activity was interrogated in TNF treated MEFs by immunoprecipitating the **IKK complex** with an anti-IKK.gamma. antibody and then measuring the capacity of this **complex** to phosphorylate a recombinant GST-I.kappa.B.alpha. (residue #1-54) substrate. This kinase assay demonstrates that **IKK** activity is indeed prolonged in A.sub.20.sup.-/- MEFs, compared to normal cells (FIG. 22B). Thus A20, itself induced by TNF, terminates TNF induced NF-.kappa.B signals by inhibiting **IKK** phosphorylation of I.kappa.B.alpha.. A20 is absolutely essential for this function, and is thus a critical regulator of inflammatory gene expression in vivo.

Detail Description Paragraph - DETX (332):

[0310] This finding indicates that A20 may either regulate translation of I.kappa.B.alpha. mRNA, or prevent degradation of newly synthesized I.kappa.B.alpha. protein. It is contemplated that A20 performs the latter function by inhibiting the phosphorylation activity of the enzyme **complex** inhibitor of kappa kinase (**IKK**), or by regulating a more proximate activation step between TNFR and **IKK**. To distinguish between these functions for A20, MEFs were treated with the proteosome inhibitor MG-132 fifteen minutes after TNF treatment. This dual treatment led to the reaccumulation of I.kappa.B.alpha. protein in A20.sup.-/- MEFs, indicating that I.kappa.B.alpha. protein is synthesized normally in the absence of A20, but is rapidly degraded by proteosome dependent pathways (FIG. 22A). As the rapid degradation of I.kappa.B.alpha. protein could be due to rapid phosphorylation of I.kappa.B.alpha. by **IKK**, an examination was done of whether **IKK** activity in TNF treated MEFs by immunoprecipitating the **IKK complex** with an anti-IKK.gamma. antibody and then measuring the capacity of this **complex** to phosphorylate a recombinant GST-I.kappa.B.alpha. (residue #1-54) substrate. This kinase assay demonstrates that **IKK** activity is indeed prolonged in A20.sup.-/- MEFs, compared to normal cells (FIG. 22B). Thus A20, itself induced by TNF, terminates TNF induced NF-.kappa.B signals by inhibiting **IKK** phosphorylation of I.kappa.B.alpha.. A20 is essential for this function--even in the presence of de novo synthesized I.kappa.B.alpha. protein--and is thus is indicated to be a critical regulator of inflammatory gene expression in immune cells.

Detail Description Paragraph - DETX (383):

[0343] To examine the role(s) of A20 in regulating interactions between critical TNFR signaling molecules, similar techniques and reagents will be used as described herein to examine the kinetics of these interactions in TNF treated A20.sup.-/- and A20.sup.+/+ MEFs. RIP recruitment to the TNFR is

thought to induce oligomerization of IKK.gamma. molecules, which in turn activate IKK.alpha./IKK.beta./IKK.gamma. ("signalosome") complexes that phosphorylate I.kappa.B.alpha.. Both RIP and IKK.gamma. are required for TNF induced NF-.kappa.B activation. Thus, RIP-IKK.gamma. interactions will be examined by immunoprecipitating IKK.gamma. from lysates of TNF treated A20.sup.-/- and A20.sup.+/+ MEFs, and analyzing these immunoprecipitates by Western blotting for the presence of RIP protein. Lysates will be assayed at 0, 15, 30, 60 and 90 minutes after TNF treatment. Aliquots of all immunoprecipitates will also be assayed for the quantity of IKK.beta. by western blot analysis to confirm comparable amounts of **IKK complex** in all samples. The kinetics of RIP-IKK.gamma. interactions will then be compared in lysates from A20.sup.-/- and A20.sup.+/+ MEFs to determine whether physiological A20 regulates the duration of RIP-IKK.gamma. association. As RIP-IKK.gamma. interactions are thought to be essential for TNF induced activation of the **IKK** "signalosome," immunoprecipitates of TNF treated MEF lysates will be analyzed for their functional capacity to activate NF-.kappa.B activity. Thus, immunoprecipitated lysates will be incubated with a GST-I.kappa.B.alpha. (aa 1-54) fusion protein and .sup.32P-ATP, and assayed for **IKK** kinase activity. Aliquots of these TNF treated MEFs will also be assayed for NF-.kappa.B activity by EMSA. Finally, to confirm that A20 directly modulates these interactions, A20.sup.-/- MEFs will be complemented with the A20 cDNA expression construct to confirm that abnormalities in these cells are solely due to the lack of A20.

Detail Description Paragraph - DETX (391):

[0349] When bound by conserved bacterial motifs, Toll like receptors (TLRs) induce NF-.kappa.B activation in one of the most evolutionarily conserved immune signaling pathways. TLR4 is preferentially expressed on innate immune cells and binds LPS. As innate immune cells expand aberrantly in A20.sup.-/- mice, and as A20.sup.-/- mice appear hypersensitive to LPS, it is possible that A20 is critical for regulating TLR4 signals. Thus, purified A20.sup.-/- macrophages and B cells will be stimulated with LPS and NF-.kappa.B responses studied in the same ways described above. If NF-.kappa.B responses persist abnormally, then these studies will be repeated in A20.sup.-/- TNF.sup.-/- cells. If A20 is essential for regulating TLR4 responses as well as TNF responses, then A20 may mediate these effects at the shared **IKK** signalosome **complex**. Alternatively, A20 may regulate distinct proteins in TNF and TLR4 induced NF-.kappa.B signaling pathways. Compiling the data above with the studies described herein will provide significant biochemical and biological insight into A20's functions in vivo.

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TITLE: Compounds and methods for modulating activation of NF-kB

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ABSTRACT:

Compositions and methods for modulating the activation of nuclear factor .kappa.B (NF-.kappa.B) are provided. The compositions comprise one or more agents that modulate ubiquitination of phosphorylated I.kappa.B.alpha. and/or I.kappa.B.beta.. Such compositions may be used for treating diseases associated with NF-.kappa.B activation. Modulating agents include human E3 ubiquitin ligases, antibodies thereto and variants thereof, as well as related proteins.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (13):

[0027] FIG. 6 is an autoradiogram illustrating the association of the ubiquitin-ligase with the I.kappa.B.alpha./NF-.kappa.B **complex,** following **IKK**-phosphorylation of I.kappa.B.alpha. at the DSGLDS (SEQ ID NOs:8 and 19) site. .sup.35S-labeled I.kappa.B.alpha./NF-.kappa.B **complex** immunopurified from non-activated cells was phosphorylated by **IKK**-2EE (where marked by + at the top), incubated with non-activated HeLa lysate as an E3 source, washed and subjected to in vitro ubiquitination in the presence of ATP.gamma.S, ubiquitin, E1, UBC5C (except where an excluded component is indicated by Abst Ub-Enz). Lanes 2-7 show phosphorylation by **IKK**; lanes 1 and 3-7 show the effect of incubation with HeLa lysate; in lane 4, a pI.kappa.B.alpha. peptide was added during the incubation with HeLa lysate: in lane 5, serine-substituted I.kappa.B.alpha. peptide was added during HeLa incubation; in lane 6, E1 was omitted from the ubiquitination stage; and in lane 7, UBC5C was omitted during ubiquitination.

Brief Description of Drawings Paragraph - DRTX (14):

[0028] FIGS. 7A and 7B illustrate the identification of I.kappa.B.alpha.-binding proteins associated with ubiquitin-ligase activity. FIG. 7A is a photograph showing Colloidal Blue staining of SDS-polyacrylamide gel samples of immunopurified fractions containing I.kappa.B.alpha./NF-.kappa.B and associated proteins. I.kappa.B.alpha./NF-.kappa.B **complex** was phosphorylated by **IKK**-2EE (lanes 2, 3) or mock-phosphorylated and used to adsorb the ubiquitin-ligase from HeLa lysate (lanes 1, 2). Molecular-size markers (.kappa.D) are indicated on the right. Proteins identified by mass-spectrometry analysis are indicated on the left. Gel-sites corresponding to the bands associated with the ubiquitin-ligase activity (p54 and p58) are marked on the left by brackets. FIG. 7B is an autoradiogram of proteins adsorbed onto pI.kappa.B.alpha./NF-.kappa.B from .sup.35S-labeled HeLa cells. Radiolabeled HeLa lysate was incubated with **IKK**-phosphorylated antibody-immobilized I.kappa.B.alpha./NF-.kappa.B **complex**. The immune-complexes were then washed, eluted and analyzed by SDS-PAGE and autoradiography. Lane 1 snows non-phosphorylated I.kappa.B.alpha./NF-.kappa.B **complex** incubated with HeLa lysate; lanes 2-4 show phosphorylated I.kappa.B.alpha./NF-.kappa.B**-complex** incubated with HeLa lysate in the absence (lane 2) or presence of pI.kappa.B.alpha.-peptide (lane 3) or serine-substituted I.kappa.Ba.alpha.-peptide (lane 4). Indicated on the left are molecular size markers (.kappa.D), Rel A and I.kappa.B.alpha. bands; indicated in the right are the four pI.kappa.B.alpha.-associated bands, three of which were displaced by the pI.kappa.B.alpha. peptide (arrows).

Detail Description Paragraph - DETX (87):

[0114] The finding that E1 and E2 specifically complemented pI.kappa.B.alpha.-conjugation of the stimulated HeLa fraction, but failed to complement a non-stimulated fraction, could be explained in several ways: a) HeLa stimulation activates a specific pI.kappa.B-ubiquitin ligase, b) HeLa stimulation modifies the substrate, thus rendering it liable to ubiquitination, or c) HeLa stimulation is necessary for modifying both the substrate and the ligase. To distinguish among these possibilities, a recombinant, constitutively active **IKK2** protein (**IKK2**-EE) was used (Mercurio et al., Science 278:860-66, 1997). This protein phosphorylates I.kappa.B.alpha. at serine

32/36 similarly to a TNF.alpha. activated **IKK-complex**.

Detail Description Paragraph - DETX (91):
[0118] 1) A ubiquitin-ligase component essential to pI.kappa.B.alpha. ubiquitination is recruited by the I.kappa.B.alpha./NF-.kappa.B **complex** from the HeLa lysate following **IKK** phosphorylation.

Detail Description Paragraph - DETX (97):
[0124] SDS-PAGE analysis of the three fractions revealed pattern-changes due to **IKK** phosphorylation or to further immuno-adsorption of I.kappa.B.alpha./NF-.kappa.B proteins, but did not discern any protein recruited to the I.kappa.B-**complex** following **IKK** phosphorylation. The complexity of the protein staining could obscure the presence of any recruited protein migrating along with an immunopurified protein. To identify the recruited protein, mass-spectrometry analysis was performed on a dozen Colloidal Blue-stained bands derived from fractions 1 and 2. This analysis revealed the presence of nearly the full spectrum of the Rel family proteins and I.kappa.B.alpha.: NF-.kappa.B1 (p105), NF-.kappa.B2 (p100), RelA (p65), p50, p49, C-Rel, I.kappa.B.alpha. and I.kappa.B.epsilon.. Only a few other proteins were co-immunoprecipitated with the I.kappa.B/NF-.kappa.B **complex,** particularly GRP78/Bip, Hsp 70 and Hsc 70.

Detail Description Paragraph - DETX (98):
[0125] To circumvent the possible masking of the putative pI.kappa.B-ubiquitin ligase, we replaced the ligase source with .sup.35S-biosynthetically-labeled HeLa lysate and traced the I.kappa.B.alpha.-associated proteins by SDS-PAGE analysis and autoradiography (FIG. 7B). In parallel, the various fractions were tested for their ubiquitin-ligase capacity. The band-pattern of the active fraction (lane 2) was compared with that of the non-active one (lane 1). Four .sup.35S-protein bands with a molecular mass of 54, 58, 61 and 64 kD were distinguished in lane 2. Some of these protein bands could represent components of the ubiquitin ligase that recognizes pI.kappa.B.alpha. directly whereas others might have associated with pI.kappa.Ba indirectly or with another component of the **IKK**-phosphorylated **complex**. To sort out the ligase component that recognizes pI.kappa.B.alpha. directly, pp12 or the control peptide p12S/E was added to the radiolabeled HeLa lysate, which was then incubated with the immuno-bound I.kappa.B.alpha./NF-.kappa- .B **complex**. A comparison of the eluted fractions showed that of the four distinctive bands present only in fraction 2, three bands were eliminated by the specific pp12 peptide (p54, p58 and p61), whereas only the 64 kD band persisted in the presence of pp12 (FIG. 7B, compare lanes 2 and 3). The control peptide did not affect the association of any of the distinctive proteins with PI.kappa.B.alpha. (lane 4). Two of the PI.kappa.B.alpha. interacting proteins, p58 and p54, were consistently present and always associated with the specific ubiquitin-ligase activity.

Detail Description Paragraph - DETX (107):
[0132] The ability of these proteins to bind pI.kappa.B.alpha. specifically and assist in its ubiquitination was examined in a cell-free system. The

I.kappa.B.alpha./NF-.kappa.B **complex** was immunopurified from HeLa cells and the immune **complex** was either phosphorylated with **IKK2**-EE or mock-phosphorylated as described above. It was then incubated with the following immobilized FLAG-tagged E3 family members immunoprecipitated from transfected 293 cells: mouse .beta.-TrCP (m.beta.-TrCP), human .beta.-TrCP (h.beta.-TrCP), human .beta.-TrCP with a deletion of the F box region residues 122-168 (.DELTA..beta.-TrCP) and the Drosophila Slimb protein. The bound material was analyzed by Western blotting with anti-I.kappa.B.alpha. and anti-FLAG antibodies. All of these proteins exclusively bound **IKK**-phosphorylated, but not mock-phosphorylated, I.kappa.B.alpha. (see FIG. 11A). However, the human and mouse .beta.-TrCP bound I.kappa.B.alpha. far better than the highly homologous Drosophila protein (compare lanes 2, 4, 6 and 8). .DELTA..beta.-TrCP bound pI.kappa.B.alpha. even better than the wild type protein, indicating that the F-box region was dispensable for binding. Furthermore, .beta.-TrCP binding was abrogated by a peptide representing the pI.kappa.B.alpha. recognition motif (pp10; DRHDS(PO.sub.3)GLDS(PO.sub.3)M (SEQ ID NO:29); see FIG. 11B, lane 3), but not by the control peptide (lane 4), specifying the site of PI.kappa.B.alpha. recognition of the conserved DS(PO.sub.3)GLDS(PO.sub.3) (SEQ ID NO:30) sequence.

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TITLE: Inhibition of interleukin-1-beta secretion by card proteins

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

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ABSTRACT:

The present invention provides isolated Pseudo-ICE and ICE-Like, functional fragments thereof, or immunogenic fragments thereof and nucleic acid molecules encoding the above polypeptides. Also provided are various methods of using these polypeptides or nucleic acid molecules in modulating apoptosis or inflammation.

---------- KWIC ---------

Detail Description Paragraph - DETX (161):

[0240] The results of the above assay indicate that Pseudo-ICE strongly activates NF-.kappa.B in a dose-dependent manner and enhances TNF-.alpha.-induced NF-.kappa.B activation (Panel A of FIG. 6). At levels of expression comparable to that of Pseudo-ICE, ICE-Like has very little effect on the basal or TNF-.alpha.-induced NF-.kappa.B activity (Panel A of FIG. 6). This suggests that NF-.kappa.B activity is unlikely to be provoked by a non-specific cellular stress mediated by transient expression of Pseudo-ICE. This stress termed the endoplasmic reticulum-overload response has been described by Pahl et al. (Pahl, Oncogene 18:6853, 1999). Finally, a kinase inactive mutant of **IKK**-.alpha. dose-dependently inhibits NF-.kappa.B activity provoked by Pseudo-ICE without modifying its expression as shown in Panel B of

FIG. 6. Combined, these results indicate that Pseudo-ICE activates NF-.kappa.B via a mechanism dependent on the **IKK complex** (Karin, Oncogene 18:6867, 1999). Since Pseudo-ICE, but not ICE-Like, interacts with the CARD-containing kinase RICK, it is possible that Pseudo-ICE induces oligomerization of RICK leading to activation of NF-.kappa.B. Oligomerization of RIP and RICK, which interact directly with **IKK**-.gamma., an essential component of the **IKK complex,** has been recently proposed as a mechanism by which upstream regulators transmit their activation signals to the **IKK complex** leading to its activation (Poyet et al., J. Biol. Chem. 275:37966, 2000; Inohara et al., J. Biol. Chem. 275:27823, 2000).

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TITLE: METHODS FOR IDENTIFYING COMPOUNDS THAT ANTAGONIZE CD40 SIGNALING

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INVENTOR-INFORMATION:

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ABSTRACT:

The present invention provides methods for identifying a molecule that antagonizes or agonizes CD40 activity by screening for molecules that modulate the binding of NEMO and CYLD. Also provided are assays useful in screening for the binding of NEMO and CYLD and databases and methods useful for analyzing the resultant information.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (6):

[0004] Triggering of CD40, such as by contact with membrane-bound or soluble CD40 ligand (CD40L), results in the stimulation of CD40-mediated cellular responses. These cellular responses can include the activation of transcription factor NF-kappaB, a ubiquitous transcription factor that is extensively utilized in cells of the immune system. NF-kappaB is normally maintained in the cytoplasm by interaction with IkappaB members (reviewed in Karin, Oncogene 18: 6867, 1999; Chen and Gosh, Oncogene 18:6845, 1999). Release of NF-kappaB, and subsequent translocation of this transcription factor to the nucleus, result from phosphorylation of IkappaB, which leads to its ubiquitinization and degradation. Phosphorylation of IkappaB is mediated by the inducible kappaB kinase (**IKK) complex** consisting of two kinases, IKKalpha, and IKKbeta, and a scaffolding protein referred to as NEMO (NF-kappaB essential modulator; Yamaoka et al., Cell 93:1231, 1998), **IKK**-gamma (Rothwarf et al., Nature 395:297, 1998), FIP3 (Fourteen K interacting protein 3; Li et al., Proc. Natl. Acad. Sci. USA 96:1042, 1999) or RAP2 (RIP-associated protein 2; WO

99/47672) (hereinafter, NEMO).

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TITLE: Human Pellino polypeptides

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ABSTRACT:

There are disclosed novel polypeptides referred to as Pellino polypeptides, as well as fragments thereof, including immunogenic peptides. DNAs encoding such polypeptides as well as methods of using such DNAs and polypeptides are also disclosed.

[0001] This application is a continuation-in-part of U.S. application Ser. No. 09/843,905, filed Apr. 27, 2001; which claims the benefit under 35 U.S.C. 119(e) of U.S. provisional application Serial No. 60/200,198, filed Apr. 28, 2000; all of which are incorporated in their entirety by reference herein.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (6):

[0005] Downstream components of the Toll signaling pathway have also been evolutionarily conserved in mammalian TLR and interleukin-1 receptor signaling pathways which culminate in nuclear translocation of the transcription factor

Nuclear Factor kappa B (NF-kB). Protein kinases of the IRAK family, close homologues of Pelle (such as IRAK and IRAK4), are recruited to the activated IL-1R or TLR receptor complexes through the adaptor protein MyD88 and undergo autophosphorylation reactions. Although MyD88 is not a strict analog of Tube, both proteins contain a so-called death domain, and Tube likely serves to mediate signal transmission between Toll and Pelle, to which it binds. IRAK subsequently interacts with another adaptor molecule TRAF6, which is homologous to the recently described D-TRAF. Signals downstream of TRAF6 appear to be divergent, and not all of them are fully understood, but one consequence, in mammalian cells, is the activation of the IkB kinase (**IKK) complex** which directly phosphorylates the inhibitory Cactus homolog IkB at two N-terminal serine residues causing its ubiquitination and degradation. Released from a cytoplasmic association with IkB, NF-kB migrates into the nucleus. Recently, a candidate for an additional intermediate in Tube-Pelle interactions was found by yeast two-hybrid screening with Pelle as a bait sequence. This protein, called Pellino, was shown to interact with catalytically-competent Pelle, but not with a mutant form of Pelle that lacked kinase activity. Although a function for Pellino was not addressed in this study, it was suggested that it could either stabilize the activated form of Pelle, or mediate an interaction with downstream Pelle substrates.

Detail Description Paragraph - DETX (160):

[0211] Biochemical and genetic studies have postulated a model for the IL-1-mediated signaling pathway (FIG. 1). Upon IL-1 stimulation, the adaptor molecules MyD88 and Tollip are recruited to the IL-1 receptor **complex,** which then recruits IRAK4 and IRAK. IRAK is hyperphosphorylated, mediating the recruitment of TRAF6 to the receptor **complex (Complex** I). IRAK-TRAF6 then leaves **Complex** I to interact with pre-associated TAK1, TAB1, and TAB2 on the membrane, resulting in the formation of IRAK-TRAF6-TAK1-TAB1-TAB2 (**Complex** II). The formation of **Complex** II leads to the phosphorylation of TAK1 and TAB2, which facilitates the dissociation of TRAF6-TAK1-TAB1-TAB2 (**Complex** III) from IRAK and consequent translocation of **Complex** III to the cytosol. The formation of **Complex** III and its interaction with additional factors in the cytosol lead to the activation of TAK1. Activation of TAK1 leads to the activation of both **IKK** and MKK6, resulting in activation of NF-kB and JNK, respectively. Phosphorylated IRAK remains on the membrane and eventually is ubiquitinated and degraded.

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DOCUMENT-IDENTIFIER: US 20030144303 A1

TITLE: Aminopyrimidine and aminopyridine anti-inflammation agents

PUBLICATION-DATE: July 31, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 288968

DATE FILED: November 6, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60338312 20011107 US

US-CL-CURRENT: 514/256, 424/145.1 , 514/11 , 514/171 , 514/251 , 514/291 , 514/340 , 514/341 , 514/342 , 544/333 , 546/269.7 , 546/272.4 , 546/272.7

ABSTRACT:

Aminopyrimidine and aminopyridine (I) compounds, compositions and methods useful in the treatment of inflammatory, metabolic or malignant conditions, are provided herein. 1

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] The present invention claims the priority benefit under Title 35 U.S.C. 119(e) of U.S. Provisional Application Serial No. 60/338,312, filed Nov. 7, 2001, the disclosure of which is herein incorporated by reference This application incorporates by reference the disclosure of pending U.S. patent application Ser. No. 10/004,287, filed Oct. 23, 2001, titled "Anti-inflammation Agents," inventors Michelle F. Browner, et al.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):
[0005] In its inactive state, the NF-kB heterodimer is held in the cytoplasm

by association with inhibitory IkB proteins. Recently, the three-dimensional structure of a NF-kB/IkB ternary **complex** has been solved (Huxford et al, Cell, 95, 759 (1998); Jacobs et al, Cell, 95, 749 (1998)). When cells are treated with the appropriate stimuli, such as IL-1 or TNF, intracellular signal transduction pathways are activated that lead to the eventual phosphorylation of IkB proteins on two specific residues (serines 32 and 36 in IkB-alpha, serines 19 and 23 in IkB-beta). Mutation of one or both of these serine residues renders IkB resistant to cytokine-induced phosphorylation. This signal-induced phosphorylation targets IkB for ubiquitination and proteosome-mediated degradation, allowing nuclear translocation of NF-kB (Thanos and Maniatis, Cell, 80, 529 (1995)). The only regulated step in the IkB degradation pathway is the phosphorylation of IkB by IkB kinases (**IKK**) (Yaron et al, EMBO J. 16, 6486 (1997)).

Summary of Invention Paragraph - BSTX (6):

[0006] Several intermediate steps in the TNF- and IL-1-activated signaling pathways that result in IkB phosphorylation have been elucidated in recent years. The protein kinases MEKK1 and MLK3 have been implicated in the induction of **IKK** activity (Malinin et al, Nature, 385, 540 (1997); Song et al, Proc. Natl. Acad. Sci. USA, 94, 9792 (1997); Lee et al, Proc. Natl. Acad. Sci. USA. 95, 9319 (1998); Hehner et al, Mol. Cell. Biol. 20, 2556 (2000); Wang et al, Nature, 412, 346 (2001)). While the specific details remain somewhat unclear regarding how these or other intermediate proteins may interact with and/or stimulate **IKK** activity in cells, significant progress has been made in elucidating the enzymes responsible for IkB phosphorylation. Two **IKK** enzymes, generally referred to as either **IKK**-alpha and **IKK**-beta (Woronicz et al, Science, 278, 866 (1997); Zandi et al, Cell, 91, 243 (1997)) or **IKK-1 and IKK-2** (Mercurio et al, Science, 278, 860 (1997)) have been discovered. Both forms of **IKK** can exist as homodimers and as **IKK**-alpha/**IKK**-beta heterodimers. Another recently discovered component of the IkB kinase **complex** is a regulatory protein, known as **IKK**-gamma or NF-.kappa.B-Essential Modulator (NEMO) (Rothwarf et al, Nature, 395, 297 (1998)). NEMO does not contain a catalytic domain, and thus it appears to have no direct kinase activity and it probably serves a regulatory function. Existing data suggest that the predominant form of **IKK** in cells is an **IKK**-alpha/**IKK**-beta heterodimer associated with either a dimer or a trimer of NEMO (Rothwarf et al, Nature 395, 297 (1998)).

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DOCUMENT-IDENTIFIER: US 20030144286 A1

TITLE: Benzimidazole derivatives

PUBLICATION-DATE: July 31, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 268412

DATE FILED: October 9, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60327818 20011009 US

US-CL-CURRENT: 514/233.5, 514/303 , 514/322 , 514/395 , 544/139 , 546/118 , 546/199 , 548/307.4

ABSTRACT:

Compounds, pharmaceutical compositions and methods are provided that are useful in the treatment of inflammatory and immune-related conditions or disorders. In particular, the invention provides compounds which modulate the expression and/or function of proteins involved in inflammation, immune response regulation and cell proliferation. The subject compounds are 2-amino-imidazole derivatives.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to and claims the benefit of U.S. application Serial No. 60/327,818, filed Oct. 9, 2001, the disclosure of which is incorporated by reference herein.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (4):

[0004] IL-1 treatment of cells induces the formation of a **complex** consisting of the two IL-1 receptor chains, IL-1R1 and IL-1RAcP, and the resulting heterodimer recruits an adaptor molecule designated as MyD88 (Wesche et al. (1999) J. Biol. Chem. 274:19403-19410). MyD88 binds to a protein designated IRAK (IL-1 receptor associated kinase) (see, O'Neill et al. (1998) J. Leukoc. Biol. 63(6):650-657, Auron (1998) Cytokine Growth Factor Rev. 9(3-4):221-237 and O'Neill (2000) Biochem. Soc. Trans. 28(5)557-563, for reviews). IRAK is subsequently phosphorylated and released from the receptor **complex** to interact with a tumor necrosis factor receptor-associated factor, TRAF6, which transduces the signal to downstream effector molecules (Cao et al. (1996) Nature 383:443-446). TRAF6 can trigger the NIK/**IKK** kinase cascade to activate the transcription factor NF-.kappa.B. NF-.kappa.B regulates a number of genes that, in turn, regulate immune and inflammatory responses.

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DOCUMENT-IDENTIFIER: US 20030138811 A1

TITLE: BioMAP analysis

PUBLICATION-DATE: July 24, 2003

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APPL-NO: 10/ 236558

DATE FILED: September 5, 2002

RELATED-US-APPL-DATA:

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parent continuation-in-part-of PCT/US01/07190 20010306 US PENDING

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non-provisional-of-provisional 60195672 20000407 US

US-CL-CURRENT: 435/6, 435/325 , 435/455 , 702/20

ABSTRACT:

The involvement of an expression product in a cell in a pathway is determined by genetically modifying the cell, incubating the cell with predetermined factors in induce a physiological state and measuring parameters affected by the pathway. Changes in the levels of the parameters as a result of the presence of the expressed product indicate that the expression product is involved with the pathway.

[0001] This application is a continuation-in-part of application serial no. PCT/US01/07190, International Publication No. WO 01/67103, filed Mar. 6, 2001, and claims priority to provisional application No. 60/186,976, filed Mar. 6, 2000 and No. 60/195,672, filed Apr. 7, 2000, the entire contents of each of which is incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (218):

[0221] Multiple signaling pathways contribute to expression of endothelial cell molecules. For example, TNF-alpha and IL-1 activate the NFKB pathway resulting in increased transcription of E-selectin, ICAM-1, VCAM-1 and IL-8 by HUVEC (Collins, et al., Faseb J 1995, 9, 899-909 and FIG. 5). Upon binding to their respective cell surface receptors TNF-alpha and IL-1 induce signal transduction cascade involving multiple kinases, transcription factors and inhibitor proteins (Baeuerle, Curr Biology 1998, 8, R19-R22). The NF.kappa.B pathway is a well-studied pathway in endothelial cells. Briefly, NF.kappa.B (p65/p50 transcription factor dimer) is constitutively present in the cytoplasm of unstimulated endothelial cells in a **complex** with I.kappa.B protein, which prevents NF.kappa.B from entering the nucleus and activating gene transcription. Upon stimulation by TNF-alpha or IL-1, I.kappa.B is phosphorylated by **IKK** kinase, which in turn is activated by NIK kinase. Phosphorylated I.kappa.B is ubiquitinylated and degraded releasing the NF.kappa.B dimers, which can now move to the nucleus where they bind to NF.kappa.B binding promoter sites, and activate gene expression (reviewed in Baeurle, 1998, supra).

PGPUB-DOCUMENT-NUMBER: 20030134265

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030134265 A1

TITLE: Screening method for nucleic acids

PUBLICATION-DATE: July 17, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 168683

DATE FILED: October 24, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DE	199 62 604.9	1999DE-199 62 604.9	December 23, 1999
DE	100 36 175.7	2000DE-100 36 175.7	July 2, 2000

PCT-DATA:
APPL-NO: PCT/EP00/13132
DATE-FILED: Dec 21, 2000
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/4, 435/455 , 435/6

ABSTRACT:

The invention relates to a method for determining the activity of nucleic acid sequences and to the use of the nucleic acid sequences identified in this way for providing diagnostic and therapeutic agents.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (4):
[0060] FIG. 3 shows a diagrammatic representation of the activation of the NF.kappa.B signal transduction pathway. According to the Use Example 1, the target organism (Hela cells) secretes TNF alpha which binds to a TNF receptor

of a test organism which has been added to the screening mixture and has been transfected stably with the reporter gene. The signals then initiating from the TNF receptor activate a kinase **complex of ikK** kinases which in turn phosphorylate i.kappa.B and thus effect its release from the **complex** with NF.kappa.B. NF.kappa.B can then translocate into the nucleus where it can stimulate transcription of the NF.kappa.B-dependent gene for photinus pyralis luciferase.

Detail Description Paragraph - DETX (3):

[0071] The method of the invention comprises determination of the activity of the nucleic acid sequence in the individual populations of target organisms. If the target organism contains a reporter vector, it is possible to use changes in the activity of the reporter vector as a measure of the activity of the nucleic acid sequence to be studied. In Examples 1 to 4 and 6 below the gene product encoded by the reporter vector is the enzyme luciferase (Lui et al., Gene 202 (1977), 141-148). The report vector used were reporter plasmid constructs (Schwartz et al., Gene 88 (1990), 197-205) carrying the gene for photinus pyralis luciferase under the control of an NF.kappa.B-dependent promoter. Transcription factor NF.kappa.B is activated via a signal cascade whose starting point is tumor necrosis factor alpha (TNF-alpha). TNF-alpha is a secreted polypeptide which can bind to target cells having a TNF receptor. Binding to the receptor initiates the signal cascade in the target cells, leading to activation of NF.kappa.B. NF.kappa.B is present in the cytoplasm in inactive form, complexed with the inhibiting protein i.kappa.B. The signals coming from the TNF receptor activate a kinase **complex of ikK** kinases which in turn phosphorylate i.kappa.B and thus effect its release from the **complex** with NF.kappa.B. NF.kappa.B can now translocate into the nucleus of the cell where it is able to stimulate transcription of the NF.kappa.B-dependent gene for photinus pyralis luciferase.

PGPUB-DOCUMENT-NUMBER: 20030125361

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030125361 A1

TITLE: Substituted pyrazolyl benzenesulfamide compounds for the treatment of inflammation

PUBLICATION-DATE: July 3, 2003

INVENTOR-INFORMATION:

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Iula, Donna M.	Palatine	IL	US	
Liao, Shuyuan	Northbrook	IL	US	
Stealy, Michael A.	Libertyville	IL	US	
Weier, Richard M.	Lake Bluff	IL	US	
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Vazquez, Michael L.	Ballwin	MO	US	

APPL-NO: 10/ 247021

DATE FILED: September 19, 2002

RELATED-US-APPL-DATA:

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US-CL-CURRENT: 514/359, 514/340 , 514/361 , 514/364 , 514/367 , 514/375 , 514/393 , 514/406 , 514/411 , 548/126 , 548/150 , 548/217 , 548/257 , 548/302.1 , 548/359.1 , 548/427

ABSTRACT:

The present invention relates to substituted pyrazolyl derivatives, compositions comprising such, intermediates, methods of making substituted pyrazolyl derivatives, and methods for treating cancer, inflammation, and inflammation-associated disorders, such as arthritis.

[0001] The present application claims priority under Title 35, United States Code, .sctn.119 to U.S. Provisional application Serial No. 60/323,230, filed Sep. 19, 2001, which is incorporated by reference in its entirety as if written herein.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0004] NF-.kappa.B is a ubiquitous transcription factor that plays a prominent role in the activation of the immune system and in stress responses by regulating the transcription of many early, inducible genes including proinflammatory cytokines, adhesion molecules, growth factors, enzymes, and receptors (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342). Specificity of gene expression is determined at a cellular level by a diverse array of external stimuli such as bacterial products including LPS, as well as cytokines, most importantly tumor necrosis factor-.alpha. (TNF.alpha.) and interleukin-.beta. (IL1.beta.). Through the synergistic interaction with other transcription factors, further specificity can be achieved while maintaining enormous potential to coordinately induce a large number of functionally related genes. NF-.kappa.B is composed of homo and heterodimers of the Rel protein family and is sequestered in an inactive form in the cytoplasm by members of the I.kappa.B family of inhibitory proteins (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342). I.kappa.Bs mask the nuclear localization signal on NF-.kappa.B, preventing nuclear translocation and hence DNA binding to the promoter regions of responsive genes. Stimulation of cells with an agonist that activates NF-.kappa.B leads to a series of biochemical signals, ultimately resulting in the phosphorylation, ubiquitinylation, and degradation of I.kappa.Bs, thereby releasing NF-.kappa.B for nuclear translocation (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342). Recently, two I.kappa.B kinases (**IKK1** or IKK.alpha. and **IKK2** or IKK.beta.), which phosphorylate I.kappa.Bs and thereby initiate their degradation, have been cloned and characterized by a number of laboratories (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342). The catalytic subunits, **IKK1 and IKK2,** are similar structurally as well as enzymatically and exist as a heterodimer in a large protein **complex** referred to as the **IKK** signalsome (Regnier, C., Song, H., Gao, X., Goeddel, D., Cao, Z. and Rothe, M. (1997) Cell 90, 373-383; DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E. and Karin, M. (1997) Nature 388, 548-554; Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. and Roa, A. (1997) Science 278, 860-866; Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Woronicz, J. D., Gao, X., Cao, Z., Rothe, M. And Goeddel, D. V. (1997) Science 278, 866-869). A third protein, NEMO (IKK.gamma., IKKAP1), is a regulatory adapter protein necessary for **IKK** activation and kinase activity (Yamaoka, S., Courtois, G., Bessia, C., Whiteside, S. T., Weil, R., Agou, F., Kirk, H. E., Kay, R. J., and Ireal, A. (1998) Cell 93, 1231-1240; Rothwarf, D. M., Zandi, E., Natoli, G., Karin, M. (1998) Nature 395, 297; Mercurio, F., Murray, B. W., Shevchenko, A., Bennet, B. L., Young, D. B., Li, J. W., Pascual, G., Motiwala, A., Zhu, H., Mann, M and Manning, A. M. (1999) Mol. Cell. Biol. 2, 1526-1538). **IKK1 and IKK2** are co-expressed in most human adult tissues as well as in different developmental stages of mouse embryos (Regnier, C., Song, H., Gao, X., Goeddel, D., Cao, Z. and Rothe, M. (1997) Cell 90, 373-383; DiDonato,

J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E. and Karin, M. (1997) Nature 388, 548-554; Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. and Roa, A. (1997) Science 278, 860-866; Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Woronicz, J. D., Gao, X., Cao, Z., Rothe, M. and Goeddel, D. V. (1997) Science 278, 866-869; Hu, M. C. T., and Wang, Y. (1998) Gene 222, 31-40). This kinase **complex** appears to represent a critical, common denominator in the activation of NF-.kappa.B in a number of signal transduction pathways stimulated by a variety of agonists including cytokines, such as TNF.alpha. and IL1.beta., microbial products such as LPS and viral proteins such as TAX, as well as phorbol esters, oxidizing agents and serine/tyrosine phosphatases (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342).

Summary of Invention Paragraph - BSTX (6):

[0005] **IKK1** (also termed IKK.alpha., Regnier, C., Song, H., Gao, X., Goeddel, D., Cao, Z. and Rothe, M. (1997) Cell 90, 373-383; DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E. and Karin, M. (1997) Nature 388, 548-554; Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. And Roa, A. (1997) Science 278, 860-866) was cloned simultaneously by standard biochemical purification of the I.kappa.B kinase activity from TNF.alpha. stimulated HeLa S3 cells and by its interaction with the MAP3K, NF-.kappa.B inducing kinase (NIK), in a yeast two-hybrid screen. **IKK1** was identified as the previously cloned serine-threonine kinase, CHUK (Connelly, M. and Marcu, K. (1995) Cell. Mol. Biol. Res. 41, 537-549). **IKK1** (also termed IKK.alpha.) is an 85 kDa, 745 amino acid protein that contains an N-terminal serine/threonine kinase catalytic domain, a leucine zipper-like amphipathic helix, and a C-terminal helix-loop-helix domain. **IKK2** (also termed IKK.beta.) was also cloned by standard biochemical purification, copurifying with **IKK1** from TNF.alpha. stimulated HeLa S3 cells as well as by being identified in the public database from an EST clone with sequence homology to **IKK1** (Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B.L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. and Roa, A. (1997) Science 278, 860-866; Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Woronicz, J. D., Gao, X., Cao, Z., Rothe, M. And Goeddel, D. V. (1997) Science 278, 866-869). **IKK2** is an 87 kDa, 756 amino acid protein with the same over all topology as **IKK1** except for the addition of an 11 amino acid extension at the C-terminus. **IKK1 and IKK2** are 52% identical overall with 65% identity in the kinase domain and 44% identity in the protein interaction domains in the C-terminus. Data obtained using transient mammalian expression analysis, by in vitro translation experiments and by coexpression in a baculoviral system reveals that **IKK1 and IKK2** associate preferentially as a heterodimer through their leucine zipper motifs. Although homodimers have also been described in these systems, the heterodimer is thought to be the physiologic form of the kinase in mammalian cells (Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Li, J., Peet, G. W., Pullen, S. S., Schembri-King, J., Warren, T.C., Marcu, K. B., Kehry, M. R., Barton, R. and Jakes, S. (1998) J. Biol. Chem. 273, 30736-30741). Finally, NEMO (also termed IKK.gamma.) contains three a-helical regions including a leucine zipper,

interacts preferentially with **IKK2** and is required for activation of the heterodimeric kinase **complex** perhaps by bringing other proteins into the signalsome **complex** (Yamaoka, S., Courtois, G., Bessia, C., Whiteside, S. T., Weil, R., Agou, F., Kirk, H. E., Kay, R. J., and Ireal, A. (1998) Cell 93, 1231-1240; Rothwarf, D. M., Zandi, E., Natoli, G., Karin, M. (1998) Nature 395, 297; Mercurio, F., Murray, B. W., Shevchenko, A., Bennet, B. L., Young, D. B., Li, J. W., Pascual, G., Motiwala, A., Zhu, H., Mann, M and Manning, A. M. (1999) Mol. Cell. Biol. 2, 1526-1538).

Summary of Invention Paragraph - BSTX (8):

[0007] **IKK2** demonstrates a more potent kinase activity compared to **IKK1** using I.kappa.B.alpha. or I.kappa.B.beta. as a substrate (Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. and Roa, A. (1997) Science 278, 860-866; Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Woronicz, J. D., Gao, X., Cao, Z., Rothe, M. and Goeddel, D. V. (1997) Science 278, 866-869; Dehase, M., Hayakawa, M., Chen, Y., and Karin, M. (1999) Science 284, 309-313). Mutations of the phospho-acceptor serine residues within the MAPKK activation loop alters **IKK2** kinase activity; the serine to alanine substitutions result in decreased kinase activity whereas the serine to glutamic acid substitutions result in a constitutively active kinase. Similar alanine mutations in **IKK1** do not result in a decreased stimulation of total **IKK** activity in response to TNF.alpha. or IL1.beta. (Dehase, M., Hayakawa, M., Chen, Y., and Karin, M. (1999) Science 284, 309-313). **IKK2** being the dominant kinase activity within the **IKK complex** is further supported by the analysis of fibroblasts from mice deficient in **IKK1 or IKK2**. Fibroblasts lacking **IKK1** retain full **IKK** activity in response to cytokines and could activate NF-.kappa.B. In contrast, fibroblasts lacking **IKK2** do not exhibit **IKK** activity when stimulated with cytokines nor do they activate NF-.kappa.B. Furthermore, the phenotypes of each **IKK** knock out is unique with **IKK1** deficiency resulting in skin and skeletal defects and **IKK2** knock out being embryonic lethal due to hepatocyte apoptosis (Li, Q., Antwerp, D. V., Mercurio, F., Lee, K., and Verma, I. M. (1999) Science 284, 321-325; Takeda, K., Tekeuchi, O., Tsujimura, T., Itami, S., Adachi, O., Kawai, T., Sanjo, H., Yoshikawa, K., Terada, N, and Akira, S. (1999) Science 284, 313-316; Hu, Y., Baud, V., Delhase, M., Zhang, P., Deerinck, T., Ellisman, M., Johnson, R., and Karin, M. (1999) Science 284, 315-320; Li, Q., Lu, Q., Hwang, J. Y., Buscher, D., Lee, K., Izpisua-Belmonte, J. C., and Verma, I. M. (1999) Gene and Development 13, 1322-1328; Tanaka, M., Fuentes, M. E., Yamaguchi, K., Durnin, M. H., Dalrymple, S. A., Hardy, K. L., and Goeddel, D. V. (1999) Immunity 10, 421-429).

Detail Description Paragraph - DETX (123):

[0202] SF9 cells paste containing rhIKKs were centrifuged (100,000.times.g, 10 min) to remove debris. rhIKKs were immunoprecipitated (100 .mu.g of cell paste) from the cell supernatant using 3 .mu.g of anti-NEMO antibody (FL-419), followed by coupling to protein A sepharose beads. rhIKKs were also immunoprecipitated from affinity chromatography purified protein preparations (1 .mu.g) using anti-FLAG, anti-His or anti-NEMO antibodies (1-4 .mu.g) followed by protein A sepharose coupling. The native, human **IKK complex** was

immunoprecipitated from THP-1 cell homogenates (300 .mu.g/condition) using the anti-NEMO antibody. Immune complexes were pelleted and washed 3 times with 1 ml cold lysis buffer. Immunoprecipitated rhIKKs were chromatographed by SDS-PAGE (8% Tris-glycine) and transferred to nitrocellulose membranes (Novex) and detected by chemiluminescense (SuperSignal) using specific anti-**IKK** antibodies (**IKK**-470, **IKK1H**-744). Native **IKK2,** I.kappa.B.alpha. and NEMO proteins from cytosolic lysates (20-80 .mu.g) were separated by SDS-PAGE and visualized by chemiluminescense using specific antibodies.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030125284 A1

TITLE: Agents that modulate DNA-PK activity and methods of use thereof

PUBLICATION-DATE: July 3, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 233121

DATE FILED: August 30, 2002

RELATED-US-APPL-DATA:

child 10233121 A1 20020830

parent division-of 09848986 20010504 US PENDING

non-provisional-of-provisional 60202274 20000505 US

non-provisional-of-provisional 60262321 20010117 US

US-CL-CURRENT: 514/44, 435/6

ABSTRACT:

The present invention provides methods for modulating cell death in a eukaryotic cell, and methods for reducing DNA damage in a eukaryotic cell. The methods generally comprise modulating a biological activity of DNA-PK in a cell. The invention further provides methods of treating a condition related to cell death in an individual. The invention further provides methods of identifying agents which modulate a biological activity of DNA-PK, as well as agents identified by the methods. Methods of modulating an immune response using an identified agent are also provided.

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application serial No. 60/202,274, filed May 5, 2000, and U.S. Provisional Application serial No. 60/262,321, filed Jan. 17, 2001, both of which are incorporated by reference herein in their entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (179):

[0196] Kinase assays and immunoblotting were performed according to Li et al. ((1999) J. Exp. Med. 189:1839-1845). Briefly, BMDM were treated with ISS-ODN (5 .mu.g/ml), M-ODN (5 .mu.g/ml) on ps and po backbones as indicated, LPS-free bacterial DNA or methylated bacterial DNA (5 .mu.g/ml), LPS-free calf thymus DNA (5 .mu.g/ml), LPS (10 .mu.g/ml) or TNF.alpha. (10 ng/ml) for the indicated time periods. Cell lysates were prepared and normalized by immunoblotting (IB) with anti-IKK.alpha. polyclonal antibodies (Santa Cruz, Santa Cruz Biotech Inc., CA), anti-**IKKp** polyclonal antbodies (Santa Cruz) or anti-DNA-PKcs monoclonal antibodies (NeoMarker, Calif.). IKB kinase (**IKK) complex** or DNA-PK **complex** from 100 .mu.g of the lysates were immunoprecipitated by anti-IKK.alpha. or by anti-DNA-PKcs antibodies. The kinase activities (KA) were determined by a kinase assay using the N-terminus of IKBa (for **IKK**) or the N-terminus of p53 (for DNA-PK) as a substrate as previously described. Wang et al. (1992) Proc. Natl. Acad. Sci. USA 89:4231-4235; Li et al.(1999), supra; and Hammarsten et al. (2000) J. Biol. Chem. 275:1541-1545.

PGPUB-DOCUMENT-NUMBER: 20030125231

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030125231 A1

TITLE: Methods and compounds for the diagnosis of inflammatory disease and identification of pharmacological agents useful in the treatment of inflammatory disease

PUBLICATION-DATE: July 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Li, Xiang John	Danbury	CT	US	
Barton, Randall W.	Farmington	CT	US	

APPL-NO: 10/ 154506

DATE FILED: May 23, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60292968 20010523 US

non-provisional-of-provisional 60335474 20011115 US

non-provisional-of-provisional 60333848 20011128 US

US-CL-CURRENT: 514/1, 435/6 , 435/7.2

ABSTRACT:

Methods for the diagnosis of inflammatory bowel diseases and the identification of agents useful in the treatment of such diseases based upon the agent's effect on reducing Pim-2 expression.

RELATED APPLICATIONS

[0001] This application claims priority benefit of U.S. provisional application No. 60/292,968, filed May 23, 2001; U.S. provisional application No. 60/335,474, filed Nov. 15, 2001; and United States provisional application No. 60/333,848, filed Nov. 28, 2001.

---------- KWIC ---------

Detail Description Paragraph - DETX (36):

[0067] The present inventors have further demonstrated that the Pim-2 gene

is a bona fide NF-.kappa.B target by virtue to its response to a transdominant I.kappa.B.alpha.SR (super repressor), and that its expression may be induced by lipopolysaccharide ("LPS") (J. Biol. Chem. 276: 18579 (2001)). Studies performed by the present inventors suggest that up-regulation of Pim-2 in cells by LPS is controlled by the **IKK**/NF-.kappa.B pathway. The NF-.kappa.B signal transduction pathway involves a series of intracellular steps that promote phosphorylation and subsequent dissociation of I.kappa.B inhibitor protein from the inactive NF-.kappa.B **complex**. It is believed that liberated NF-.kappa.B translocates to the nucleus where it binds to the k enhancer element on the DNA and may activate transcription of Pim-2 gene. As the NF-.kappa.B signal transduction pathway is also induced by TNF, IL-1 and phorbol ester, these compounds as well may be used to induce Pim-2 activity.

PGPUB-DOCUMENT-NUMBER: 20030124130

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030124130 A1

TITLE: Proteomic analysis of tumors for development of consultative report of therapeutic options

PUBLICATION-DATE: July 3, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 325793

DATE FILED: December 19, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60345309 20020102 US

US-CL-CURRENT: 424/155.1, 435/7.23 , 702/19

ABSTRACT:

The present invention provides methods of identifying potential therapeutic options for treatment of a tumor, and a consultative report providing the same.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/345,309, filed Jan. 2, 2002, which is hereby incorporated by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (17):

[0014] The immunohistochemical or immunofluorescent analysis preferably comprises using a panel of at least about five, preferably at least about ten, or preferably at least about fifteen antibodies directed to different biological targets in the tumor. In some embodiments of the invention, antibodies to the following biological targets are used: Her-2/neu, Ki-67, p53, Cyclin D1, c-Jun, ER, PR, gp130, IL-6, IL-11, EGFR, TGF-.alpha., farnesyl transferase, p21.sup.ras, the latency associated peptide of TGF-.beta..sub.1, TGF-.beta.RII, bcl-2, and COX-2. Other biological targets that have been shown to play a role or are expected or suspected to play a role in tumor pathology include, but are not limited to, 14-3-3, Ab1, ACE, Adenomatous Polyposis Coli

(APC colon cancer), Afx, Akt, AP-1, Apaf-1, Apaf2, Apaf3, APC (Anaphase Promoting **Complex**), APO-1, APO-2, APO-3, Apoptosis Signal-Regulating Kinase, ASK1, AT1, ATF, ATM, ATR, Bad, Bak, Bax, Bcl-1, Bcl-w, Bcl-x1, BID, BRCA1, BRCA2, Bub1, CA2+/Calmodulin Kinase II/IV, CAD, Cadherin, Calsequestrin, CAP4, CASPASE1, CASPASE10, CASPASE11, CASPASE2, CASPASE3, CASPASE4, CASPASE6, CASPASE7, CASPASE8, CASPASE9, Cathepsin D, Caveolin, CBP, CD31(Pecam-1), CD40, CD40L, CD95, CD95L(ligand for CD95), cdc25a, cdc25b, cdc25c, Cdc34, Cdc42, Cdk2, Cdk4, Cdk6, Cdk7, Cdk8, c-Fos, chk1, Chk2/hcds1, c-myc, Cox-2, Csk, Cullin-1, Cyclin A, Cyclin B1, Cyclin, Cyclin D2, Cyclin D3, Cyclin E, Cyclin E2, cytochrome C, DAXX, DFF40, DFF45, DNA-PK, Dp-1, E2f, EGF, Eif2a, Elk-1, ErbB3, ErbB4, ERK1, ERK2, ERK3, ERK5, Estrogen Receptor, FADD, FAF1, FAK, FAP-1, Fas, FasL, Fkhrl1, FLICE, FLICE2, Fodrin, Frizzled, Fyn, gadd45, GAP, Glucocorticoid Receptor, Grb2, Growth Hormone Receptor, Gsk3b, HDAC, Her3, Her4, Histone Deacetylase 4, Histone eacetylase 5, Histone H3, HMG-1, HMG-2, Hsp90, Histone H4, ICAD, ICE, **IKKa, Ikkb, IKKg,** IL-2, IL-b 2R, Irs-1, Jak1, Jak2, Jkk1, JNK Oun n-terminal kinase), Jnk1, KSR-1 (kinase suppressor of Ras), Ku, lamin b1, Lamin a, lamin b2, Lck, MACH, MADD, Mannose 6-phosphate Receptor, MAP2, MAPK, MAPKK, MAPKKK, Mch2, Mch3, Mch4, Mch5, Mch6, mcl-1, MCM2, MCM3, MCM5, MDM-2, Mek, MEK3, MEK4, MEK6, MEK7, MIHA/XIAP, MIHB/cIAP1, MIHC/cIAP2, MLH1, MNDA, Mnk1, Mnk2, mre11, MSH2, MSk1, Mucin2, MyoD1, myt1, Nck, NFAT3, NF-Kb, nibrin, Nik, NLK, N-myc (neuronal), P/CAF, P107, P14ARF, P15, P16INK4, P19ARF, P21/waf1/cip1, P27, P300, P38 MAP Kinase, P45, P48-IFN a signaling, P51, P63, P73, Pak1, Paxillin, PCNA (Proliferating cell nuclear antigen), PDGF, PDGFR, Pdk-1, PI3K, PKB, PKC, PKR, PLCg, Poly(ADP)-Ribose Polymerase PARP), Pp130, PP2A, par, PTEN, Pyk2, Rab9, Rac, RAD50, Rad51, Rad9, Raf1, RAIDD, Ral, Ras, Rb, Replication protein A, Rho a, RIP, Rsk-1, Rsk-2, Rsk-3, SAPK (stress activated inase), She, Skp2, Smac/Diablo, SMAD1, SMAD2, SMAD3, Smad4, SnoN, SODD, Sos-1, Src, STAT1, STAT3, Stat5, STAT6, Survivin, Syk, TAB1, TAK1, Talin, Tcf4, TGFb, TNFR, TNFR1, TNFR2, TNFB, TNFa, TRADD, TRAF1, TRAF2, Traf-3, TRAF3, TRAF4, TRAF5, TRAF6, Trail,TyK2, VDAC, weel, Wilm's Tumor Protein-1 (WT-1), wnt, b-Catenin, and b-TRCP. Antibodies to these biological targets, and in some instances to their phosphorylated state, are readily available from private or commercial sources such as, for example, Oncogene Research Products (San Diego, Calif.), Calbiochem (San Diego, Calif.), Santa Cruz Biotechnology, Inc. (Santa Cruz, Calif.), and Cell Signaling Technology, Inc. (Beverly Mass.).

PGPUB-DOCUMENT-NUMBER: 20030119720

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030119720 A1

TITLE: Oligopeptide treatment of anthrax

PUBLICATION-DATE: June 26, 2003

INVENTOR-INFORMATION:

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Benner, Robert	Barendrecht		NL	

APPL-NO: 10/ 029206

DATE FILED: December 21, 2001

RELATED-US-APPL-DATA:

child 10029206 A1 20011221

parent continuation-in-part-of 09821380 20010329 US PENDING

US-CL-CURRENT: 514/2

ABSTRACT:

The invention relates to the modulation of gene expression in a cell, also called gene control, in particular in relation to the treatment of anthrax. The invention provides a method for modulating expression of a gene in a cell comprising providing the cell with a signaling molecule comprising a peptide or functional analogue thereof.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation in part of U.S. Ser. No. 09/821,380 filed on Mar. 29, 2001, the contents of which are incorporated by this reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (49):

[0047] NF-.kappa.B represents a group of structurally related and evolutionarily conserved gene transcription factors. So far, five mammalian NF-.kappa.B proteins named Rel (c-Rel), RelA (p65), RelB, NF-.kappa.B (p50 and its precursor p105), and NF-.kappa.B (p52 and it precursor p100) have been described. NF-.kappa.B proteins can exist as homo- or heterodimers, and

although most NF-.kappa.B dimers are activators of transcription, the p50/p50 and p52/p52 homodimers often repress the transcription of their target genes. In Drosophila, three NF-.kappa.B homologs named Dorsal, Dif, and Relish have been identified and characterized. Structurally, all NF-.kappa.B/Rel proteins share a highly conserved NH.sub.2-terminal Rel homology domain (RHD) that is responsible for DNA binding, dimerization, and association with inhibitory proteins known as I.kappa.BS. In resting cells, NF-.kappa.B/Rel dimers are bound to I.kappa.Bs and retained in an inactive form in the cytoplasm. Like NF-.kappa.B, IkBs are also members of a multigene family containing seven known mammalian members including I.kappa.B.alpha., I.kappa.B.beta., I.kappa.b.gamma., I.kappa.B.epsilon., Bcl-3, the precursor Rel-proteins, p100, and p105, and one Drosophila I.kappa.B named Cactus. The I.kappa.B family is characterized by the presence of multiple copies of ankyrin repeats, which are protein-protein interaction motifs that interact with NF-.kappa.B via the RHD. Upon appropriate stimulation, I.kappa.B is phosphorylated by I.kappa.B kinases (**IKKs**), polyubiquitinated by an ubiquitin ligase **complex,** and degraded by the 26S proteosome. Consequently, NF-.kappa.B is released and translocates into the nucleus to initiate gene expression.

Summary of Invention Paragraph - BSTX (63):

[0061] NF-kappaB/Rel proteins are a group of structurally related and evolutionarily conserved proteins (Rel). Well known are c-Rel, RelA (p65), RelB, NF-kappaB1 (p50 and its precursor p105), and NF-kappaB2 (p52 and its precursor p10). Most NF-kappaB dimers are activators of transcription; p50/p50 and p52/p52 homodimers repress the transcription of their target genes. All NF-kappaB/Rel proteins share a highly conserved NH2-terminal Rel homology domain (RHD). RHD is responsible for DNA binding, dimerization, association with inhibitory proteins known as IkappaBs. In resting cells, NF-kappaB/Rel dimers are bound to IkappaBs and retained in an inactive form in the cytoplasm. IkappaBs are members of a multigene family (IkappaBalpha, IkappaBbeta, IkappaBgamma, IkappaBepsilon, Bcl-3, the precursor Rel-proteins, p100 and p105. Presence of multiple copies of ankyrin repeats to interact with NF-kappaB via the RHD (protein-protein interaction. Upon appropriate stimulation, IkappaB is phosphorylated by IkappaB Kinase (**IKKs**), polyubiquitinated by ubiquitin ligase **complex,** and degraded by the 26S proteosome. NF-kappaB is released and translocates into nucleus to initiate gene expression.

PGPUB-DOCUMENT-NUMBER: 20030119017

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030119017 A1

TITLE: Enzymatic nucleic acid treatment of diseases or conditions related to levels of IKK-gamma and PKR

PUBLICATION-DATE: June 26, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 156306

DATE FILED: May 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60294412 20010529 US

US-CL-CURRENT: 435/6, 514/44 , 536/23.1

ABSTRACT:

The present invention relates to nucleic acid molecules, including antisense and enzymatic nucleic acid molecules, such as hammerhead ribozymes, DNAzymes, allozymes, aptamers, decoys and siRNA (RNAi), which modulate the expression or function of IKK genes, such as IKK-gamma, IKK-alpha, or IKK-beta, and PKR genes.

[0001] This patent application claims priority from U.S.S. No. 60/294,412, filed May 29, 2001, entitled `ENZYMATIC NUCLEIC ACID TREATMENT OF DISEASES OR CONDITIONS RELATED TO LEVELS OF IKK-GAMMA AND PKR." This application is hereby incorporated by reference herein in its entirety including the drawings.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (17):

[0016] The **IKK complex** that sequesters NFKB in the cytoplasm comprises IkappaB (I.kappa.B) proteins (I.kappa.B-alpha, I.kappa.B-beta, I.kappa.B-epsilon, p105, and p100). The phosphorylation of I.kappa.B proteins results in the release of NFKB from the I.kappa.B **complex** which is transported to the nucleus via the unmasking of nuclear translocation signals. Phosphorylation marks IkB proteins for ubiquitination and degradation via the proteosome pathway. Most NFKB inducing stimuli initiate activation of an I.kappa.B kinase (**IKK) complex** that contains two catalytic subunits, **IKK**-alpha

(IKK1) and IKK-beta (IKK2), that phosphorylate I.kappa.B-alpha and I.kappa.B-beta, with IKK-beta playing a predominant role in pro-inflammatory signaling. In addition to the two kinases, the IKK complex contains regulatory subunits, including IKK-gamma (NEMOAIKKAPi). IKK-gamma is a protein that is critical for the assembly of the IKK complex. IKK-gamma directly binds to IKK-beta and is required for activation of NFKB, for example by TNF-alpha, IL-1-beta, lipopolysaccharide, phorbol 12-myristate 13-acetate, the human T-cell lymphotrophic virus (HTLV-1), or double stranded RNA. Genomic rearrangements in IKK-gamma have been shown to impair NFKB activation and result in incontinentia pigmenti. Additional proteins that associate with the IKK complex include, MEK kinase (MEKKI), NFKB inducing kinase (NIK), receptor interacting protein (RIP), protein kinase CK2, and IKK-associated protein (IKAP), which appears to be associated with the I.kappa.B Kinase (IKK) complex, but does not appear to be an integral component of the tripartate IKK complex as does IKK-gamma (Krappmann et al., 2001, J. Biol. Chem., 275, 29779-87).

Detail Description Paragraph - DETX (1):

[0066] The invention features nucleic acid molecules, for example enzymatic nucleic acid molecules, antisense nucleic acid molecules, 2,5-A chimeras, decoys, double stranded RNA, triplex oligonucleotides, and/or aptamers, and methods to modulate gene expression, for example, genes encoding a member of the I.kappa.B kinase IKK complex, such as IKK-alpha (p1), IKK-beta (I), or IKK-gamma (IKKy) and/or a protein kinase PKR protein. In particular, the instant invention features nucleic-acid based molecules and methods to modulate the expression of IKK-gamma (IKKy) and protein kinase PKR.

Detail Description Paragraph - DETX (2):

[0067] The invention features one or more enzymatic nucleic acid-based molecules and methods that independently or in combination modulate the expression of gene(s) encoding a member of the I.kappa.B kinase IKK complex or PKR. In particular embodiments, the invention features nucleic acid-based molecules and methods that modulate the expression of a member of the I.kappa.B kinase IKK complex, for example IKK-alpha (IKKI), IKK-beta (IKK2), or IKK-gamma (IKKy) and/or a protein kinase PKR protein, such as IKK-alpha (IKK1) gene (Genbank Accession No. NM.sub.--001278); 1-beta (IKK2) gene, for example (Genbank Accession No.AF080158), IKK-1:5 gamma (IKK.gamma.) gene, for example (Genbank Accession No. NM 003639), and protein kinase PKR gene, for example (Genbank Accession No. NM 002759).

Detail Description Paragraph - DETX (3):

[0068] The description below of the various aspects and embodiments is provided with reference to the exemplary IKK-gamma and PKR genes. IKK-gamma is also known as NEMO/IKKAP1. However, the various aspects and embodiments are also directed to other genes which encode other subunits of the IKK complex, such as IKK-alpha (IKK1) or IKK-beta (IKK2). Those additional genes can be analyzed for target sites using the methods described for IKK-gamma or PKR. Thus, the inhibition and the effects of such inhibition of the other genes can be performed as described herein.

Detail Description Paragraph - DETX (40):

[0105] By "**IKK**-gamma proteins" is meant, a peptide or protein comprising a **IKK**-gamma or NEMO/IKKAP1 component of the **IKK complex,** for example a regulatory **IKK** subunit involved in the assembly of the high molecular weight **IKK complex** and/or induction of NFKB.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030114432 A1

TITLE: Substituted pyrazolyl compounds for the treatment of inflammation

PUBLICATION-DATE: June 19, 2003

INVENTOR-INFORMATION:

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Hagen, Timothy J.	Gurnee	IL	US	
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RELATED-US-APPL-DATA:

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US-CL-CURRENT: 514/183, 514/210.21 , 514/217.09 , 514/217.1 , 514/291 , 514/322 , 514/362 , 514/363 , 514/366 , 514/387 , 514/393 , 540/599 , 546/199 , 546/82 , 546/83 , 548/126 , 548/151 , 548/218 , 548/302.1

ABSTRACT:

The present invention relates to substituted pyrazolyl derivatives, compositions comprising such, intermediates, methods of making substituted pyrazolyl derivatives, and methods for treating cancer, inflammation, and inflammation-associated disorders, such as arthritis.

[0001] The present application claims priority under Title 35, United States Code, .sctn.119 to U.S. Provisional application Serial No. 60/323,297, filed Sep. 19, 2001, and U.S. Provisional application Serial No. 60/383,226, filed May 24, 2002, which are incorporated by reference in their entirety as if written herein.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0004] NF-.kappa.B is a ubiquitous transcription factor that plays a prominent role in the activation of the immune system and in stress responses by regulating the transcription of many early, inducible genes including proinflammatory cytokines, adhesion molecules, growth factors, enzymes, and receptors (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342). Specificity of gene expression is determined at a cellular level by a diverse array of external stimuli such as bacterial products including LPS, as well as cytokines, most importantly tumor necrosis factor-.alpha. (TNF.alpha.) and interleukin-.beta. (IL1.beta.). Through the synergistic interaction with other transcription factors, further specificity can be achieved while maintaining enormous potential to coordinately induce a large number of functionally related genes. NF-.kappa.B is composed of homo and heterodimers of the Rel protein family and is sequestered in an inactive form in the cytoplasm by members of the I.kappa.B family of inhibitory proteins (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342). I.kappa.Bs mask the nuclear localization signal on NF-.kappa.B, preventing nuclear translocation and hence DNA binding to the promoter regions of responsive genes. Stimulation of cells with an agonist that activates NF-.kappa.B leads to a series of biochemical signals, ultimately resulting in the phosphorylation, ubiquitinylation, and degradation of I.kappa.BS, thereby releasing NF-.kappa.B for nuclear translocation (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342). Recently, two I.kappa.B kinases (**IKK1** or IKK.alpha. and **IKK2** or IKK.beta.), which phosphorylate I.kappa.Bs and thereby initiate their degradation, have been cloned and characterized by a number of laboratories (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342). The catalytic subunits, **IKK1 and IKK2,** are similar structurally as well as enzymatically and exist as a heterodimer in a large protein **complex** referred to as the **IKK** signalsome (Regnier, C., Song, H., Gao, X., Goeddel, D., Cao, Z. and Rothe, M. (1997) Cell 90, 373-383; DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E. and Karin, M. (1997) Nature 388, 548-554; Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. and Roa, A. (1997) Science 278, 860-866; Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Woronicz, J. D., Gao, X., Cao, Z., Rothe, M. And Goeddel, D. V. (1997) Science 278, 866-869). A third protein, NEMO (IKK.gamma., IKKAP1), is a regulatory adapter protein necessary for **IKK** activation and kinase activity (Yamaoka, S., Courtois, G., Bessia, C., Whiteside, S. T., Weil, R., Agou, F., Kirk, H. E., Kay, R. J., and Ireal, A. (1998) Cell 93, 1231-1240; Rothwarf, D. M., Zandi, E., Natoli, G., Karin, M. (1998) Nature 395, 297; Mercurio, F., Murray, B. W., Shevchenko, A., Bennet, B. L., Young, D. B., Li, J. W., Pascual, G., Motiwala, A., Zhu, H., Mann, M and Manning, A. M. (1999) Mol. Cell. Biol. 2, 1526-1538). **IKK1 and IKK2** are co-expressed in most human adult tissues as well as in different developmental stages of mouse embryos (Regnier, C., Song, H.,

Gao, X., Goeddel, D., Cao, Z. and Rothe, M. (1997) Cell 90, 373-383; DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E. and Karin, M. (1997) Nature 388, 548-554; Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. and Roa, A. (1997) Science 278, 860-866; Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Woronicz, J. D., Gao, X., Cao, Z., Rothe, M. and Goeddel, D. V. (1997) Science 278, 866-869; Hu, M. C. T., and Wang, Y. (1998) Gene 222, 31-40). This kinase **complex** appears to represent a critical, common denominator in the activation of NF-.kappa.B in a number of signal transduction pathways stimulated by a variety of agonists including cytokines, such as TNF.alpha. and IL1.beta., microbial products such as LPS and viral proteins such as TAX, as well as phorbol esters, oxidizing agents and serine/tyrosine phosphatases (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342).

Summary of Invention Paragraph - BSTX (6):

[0005] **IKK1** (also termed IKK.alpha.; Regnier, C., Song, H., Gao, X., Goeddel, D., Cao, Z. and Rothe, M. (1997) Cell 90, 373-383; DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E. and Karin, M. (1997) Nature 388, 548-554; Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. And Roa, A. (1997) Science 278, 860-866) was cloned simultaneously by standard biochemical purification of the I.kappa.B kinase activity from TNF.alpha. stimulated HeLa S3 cells and by its interaction with the MAP3K, NF-.kappa.B inducing kinase (NIK), in a yeast two-hybrid screen. **IKK1** was identified as the previously cloned serine-threonine kinase, CHUK (Connelly, M. and Marcu, K. (1995) Cell. Mol. Biol. Res. 41, 537-549). **IKK1** (also termed IKK.alpha.) is an 85 kDa, 745 amino acid protein that contains an N-terminal serine/threonine kinase catalytic domain, a leucine zipper-like amphipathic helix, and a C-terminal helix-loop-helix domain. **IKK2** (also termed IKK.beta.) was also cloned by standard biochemical purification, copurifying with **IKK1** from TNF.alpha. stimulated HeLa S3 cells as well as by being identified in the public database from an EST clone with sequence homology to **IKK1** (Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. and Roa, A. (1997) Science 278, 860-866; Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Woronicz, J. D., Gao, X., Cao, Z., Rothe, M. And Goeddel, D. V. (1997) Science 278, 866-869). **IKK2** is an 87 kDa, 756 amino acid protein with the same over all topology as **IKK1** except for the addition of an 11 amino acid extension at the C-terminus. **IKK1 and IKK1** are 52% identical overall with 65% identity in the kinase domain and 44% identity in the protein interaction domains in the C-terminus. Data obtained using transient mammalian expression analysis, by in vitro translation experiments and by coexpression in a baculoviral system reveals that **IKK1 and IKK2** associate preferentially as a heterodimer through their leucine zipper motifs. Although homodimers have also been described in these systems, the heterodimer is thought to be the physiologic form of the kinase in mammalian cells (Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Li, J., Peet, G. W., Pullen, S. S., Schembri-King, J., Warren, T. C., Marcu, K. B., Kehry, M. R., Barton, R. and Jakes, S. (1998) J. Biol. Chem. 273, 30736-30741).

Finally, NEMO (also termed IKK.gamma.) contains three .alpha.-helical regions including a leucine zipper, interacts preferentially with **IKK2** and is required for activation of the heterodimeric kinase **complex** perhaps by bringing other proteins into the signalsome **complex** (Yamaoka, S., Courtois, G., Bessia, C., Whiteside, S. T., Weil, R., Agou, F., Kirk, H. E., Kay, R. J., and Ireal, A. (1998) Cell 93, 1231-1240; Rothwarf, D. M., Zandi, E., Natoli, G., Karin, M. (1998) Nature 395, 297; Mercurio, F., Murray, B. W., Shevchenko, A., Bennet, B. L., Young, D. B., Li, J. W., Pascual, G., Motiwala, A., Zhu, H., Mann, M and Manning, A. M. (1999) Mol. Cell. Biol. 2, 1526-1538).

Summary of Invention Paragraph - BSTX (8):

[0007] **IKK2** demonstrates a more potent kinase activity compared to **IKK1** using I.kappa.B.alpha. or I.kappa.B.beta. as a substrate (Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. and Roa, A. (1997) Science 278, 860-866; Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Woronicz, J. D., Gao, X., Cao, Z., Rothe, M. and Goeddel, D. V. (1997) Science 278, 866-869; Dehase, M., Hayakawa, M., Chen, Y., and Karin, M. (1999) Science 284, 309-313). Mutations of the phospho-acceptor serine residues within the MAPKK activation loop alters **IKK2** kinase activity; the serine to alanine substitutions result in decreased kinase activity whereas the serine to glutamic acid substitutions result in a constitutively active kinase. Similar alanine mutations in **IKK1** do not result in a decreased stimulation of total **IKK** activity in response to TNF.alpha. or UL1.beta. (Dehase, M., Hayakawa, M., Chen, Y., and Karin, M. (1999) Science 284, 309-313). **IKK2** being the dominant kinase activity within the **IKK complex** is further supported by the analysis of fibroblasts from mice deficient in **IKK1 or IKK2**. Fibroblasts lacking **IKK1** retain full **IKK** activity in response to cytokines and could activate NF-.kappa.B. In contrast, fibroblasts lacking **IKK2** do not exhibit **IKK** activity when stimulated with cytokines nor do they activate NF-.kappa.B. Furthermore, the phenotypes of each **IKK** knock out is unique with **IKK1** deficiency resulting in skin and skeletal defects and **IKK2** knock out being embryonic lethal due to hepatocyte apoptosis (Li, Q., Antwerp, D. V., Mercurio, F., Lee, K., and Verma, I. M. (1999) Science 284, 321-325; Takeda, K., Tekeuchi, O., Tsujimura, T., Itami, S., Adachi, O., Kawai, T., Sanjo, H., Yoshikawa, K., Terada, N, and Akira, S. (1999) Science 284, 313-316; Hu, Y., Baud, V., Delhase, M., Zhang, P., Deerinck, T., Ellisman, M., Johnson, R., and Karin, M. (1999) Science 284, 315-320; Li, Q., Lu, Q., Hwang, J. Y., Buscher, D., Lee, K., Izpisua-Belmonte, J. C., and Verma, I. M. (1999) Gene and Development 13, 1322-1328; Tanaka, M., Fuentes, M. E., Yamaguchi, K., Dumin, M. H., Dalrymple, S. A., Hardy, K. L., and Goeddel, D. V. (1999) Immunity 10, 421-429).

Detail Description Paragraph - DETX (205):

[0279] SF9 cells paste containing rhIKKs were centrifuged (100,000.times.g, 10 min) to remove debris. rhIKKs were immunoprecipitated (100 .mu.g of cell paste) from the cell supernatant using 3 .mu.g of anti-NEMO antibody (FL-419), followed by coupling to protein A sepharose beads. rhIKKs were also immunoprecipitated from affinity chromatography purified protein preparations (1 .mu.g) using anti-FLAG, anti-His or anti-NEMO antibodies (1-4 .mu.g)

followed by protein A sepharose coupling. The native, human **IKK c mplex** was immunoprecipitated from THP-1 cell homogenates (300 .mu.g/condition) using the anti-NEMO antibody. Immune complexes were pelleted and washed 3 times with 1 ml cold lysis buffer. Immunoprecipitated rhIKKs were chromatographed by SDS-PAGE (8% Tris-glycine) and transferred to nitrocellulose membranes (Novex) and detected by chemiluminescense (SuperSignal) using specific anti-**IKK** antibodies (**IKK2H**-470, **IKK1H**-744). Native **IKK2,** I.kappa.B.alpha. and NEMO proteins from cytosolic lysates (20-80 .mu.g) were separated by SDS-PAGE and visualized by chemiluminescense using specific antibodies.

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ABSTRACT:

Novel CARD-9, CARD-10, or CARD-11 polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated CARD-9, CARD-10, or CARD-11 proteins, the invention further provides CARD-9, CARD-10, or CARD-11, fusion

proteins, antigenic peptides and anti-CARD-9, CARD-10, or CARD-11 antibodies. The invention also provides CARD-9, CARD-10, or CARD-11 nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a CARD-9, CARD-10, or CARD-11 gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 09/798,412, filed Mar. 2, 2001, which is a continuation-in-part of U.S. application Ser. No. 09/728,260, filed Dec. 1, 2000, which is a continuation-in-part of U.S. application Ser. No. 09/685,791, filed Oct. 10, 2000, which is a continuation-in-part of U.S. application Ser. No. 09/513,904, filed Feb. 25, 2000, which is a continuation-in-part of application Ser. No. 09/507,533, filed Feb. 18, 2000, which claimed priority from provisional application serial No. 60/168,780, filed Dec. 3, 1999. The content of each of these applications is herein incorporated by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):

[0009] Nuclear factor-.kappa.B (NF-.kappa.B) is a transcription factor that is expressed in many cell types and activates genes that have NF-.kappa.B sites in their promoters. Molecules that regulate NF-.kappa.B activation play a critical role in both apoptosis and in the stress-response of cells. With respect to stress-reponse, NF-.kappa.B activates genes that control immune defense mechanisms and inflammation. The CARD-containing proteins RICK, CARD-4 and Bcl-10 also induce activation of the NF-.kappa.B transcription factor suggesting that CARD/CARD signaling complexes regulate activation of the **IKK complex** (Inohara et al. 1998 Proc. Natl. Acad Sci. USA 273:12296; Bertin et al. 1999 J. Biol. Chem. 224:12955; Willis et al. 1999 Cell 96: 33). In unstimulated cells, NF-?B is found sequestered in the cytoplasm through interactions with inhibitory I.kappa.B proteins. Inhibition is relieved by the phosphorylation and proteosomal degradation of I.kappa.B proteins by proinflammatory cytokines. Phosphorylation is mediated by the **IKK complex** which is comprised of at least three major proteins: two kinases designated IKK.alpha. and IKK.beta. that directly phosphorylate the I.kappa.B inhibitory proteins, and a noncatalytic subunit called IKK.gamma. that functions to link the **IKKs** to upstream regulatory molecules (Zhang et al., 2000). Recently, RICK has been found to function as upstream regulatory molecules of the **IKK complex** (Inohara et al. 2000 J. Biol. Chem. 275:27823). RICK interacts directly with IKK.gamma. suggesting that it functions as signaling adaptor between the **IKK complex** and an upstream CARD-containing NF-.kappa.B activator. Indeed, CARD-4 forms a CARD/CARD signaling **complex** with RICK that induces activation of the **IKK complex** and the subsequent release of NF-.kappa.B (Bertin et al. 1999 J. Biol. Chem. 224:12955; Inohara et al. 1999 J. Biol. Chem. 274:14566; Inohara et al. 2000 J. Biol. Chem. 275:27823).

Detail Description Paragraph - DETX (78):

[0210] These results, taken with the finding of a direct interaction between CARD-9 and Bcl-10 suggest that CARD-9 is a specific regulator of Bcl-10 function. CARD-9 could play a role as an upstream signaling molecule that recruits Bcl-10 through CARD/CARD interactions. The resulting signaling **complex** may interact directly or indirectly with components of the **IKK complex** resulting in its activation, e.g., through oligomerization of IKK.gamma.. Indeed the data described above data shows that both CARD-9 and Bcl-10 form large oligomeric complexes (filaments) when overexpressed in mammalian cells. Furthermore, enforced oligomerization of the C-terminus of Bcl-10/CLAP is thought to induce NF-.kappa.B activation, suggesting that the CARD domain of Bcl-10 functions as an oligomerization domain that transduces the activation signal to the **IKK complex** through its C-terminal domain. The ability of CARD-9 to form a **complex** with Bcl-10 via CARD/CARD interactions supports the idea that Bcl-10 functions as an adaptor between the effector **IKK complex** and the proximal signaling complexes that interact with CARD-9. Signaling molecules upstream of CARD-9 are predicted to transduce their signals to Bcl-10 through direct interactions with the C-terminal coiled-coil domain of CARD-9. Taken together, these results identify CARD-9 as an important mediator of NF-.kappa.B signaling through Bcl-10.

Detail Description Paragraph - DETX (81):

[0213] These studies showed that when CARD-11 is expressed in 293T cells, NF-kB activity is induced 20-to 40-fold compared to empty vector (FIG. 20A). NF-kB signaling occurred through the **IKK complex** since dominant-negative versions of **IKK-g and IKK-b** blocked the ability of CARD-11 to induce NF-kB activity (data not shown). To determine the role of individual domains in NF-kB signaling, a series of N- and C-terminal truncation mutants of CARD-11 were constructed (FIG. 20B). The N-terminal CARD of CARD-11 was essential for NF-kB signaling since deletion of this domain eliminated the induction of NF-kB activity (FIG. 20C). Immunoblot analysis revealed that the mutant proteins were expressed at levels similar to wt protein indicating that loss of function was not due to reduced levels of expression. In contrast, the C-terminal PDZ, SH3 and GUK domains were not required for NF-kB signaling since deletion of these domains had no effect on the ability of CARD-11 to induce NF-kB activity. However, a CARD-11 mutant lacking its C-terminal PDZ, SH3 and GUK domains induced NF-kB activity to levels 4-to 5-fold greater than that obtained with wt protein (FIG. 20C). Thus, the C-terminal domains may function to negatively regulate induction of NF-kB signaling by CARD-11.

Detail Description Paragraph - DETX (90):

[0222] CARD-11 is a specific regulator of Bcl-10 function. The finding that CARD-11 binds to Bcl-10 through a CARD/CARD interaction suggests that this molecule functions as upstream activator of Bcl-10. As discussed above, CARD-9 also binds to the CARD activation domain of Bcl-10 and signals NF-kB activation. Thus, CARD11 and CARD-9 constitute a subclass of CARD proteins that may function to transduce upstream stimuli to the activation of Bcl-10 and NF-kB. In response to upstream signals, the coiled-coil domains could mediate self-association of CARD-11 resulting in the aggregation and activation of Bcl-10. Bcl-10 might then engage and oligomerize **IKKg** resulting in the

activation of the **IKK complex** and NF-kB (Inohara et al. 1999 J. Biol. Chem. 274:14566; Poyet et al., 1999). Thus, CARD-11 could function in a manner analogous to Apaf-1 and CARD-4 that function as upstream regulators to induce oligomerization and activation of their respective downstream CARD binding partners. The data showing that CARD-11 induces the phosphorylation of Bcl-10 suggests that signal transduction may involve the participation of a serine/threonine kinase. The C-terminal PDZ/SH3/GUK domains of CARD-11 may function in an analogous manner to the C-terminal LRR domain of CARD-4 and the WD-40 domain of Apaf-1 to regulate protein activation by upstream signals. PDZ/SH3/GUK domains identify MAGUK family members, a class of proteins that associate with the plasma membrane (Fanning and Anderson, 1999 Curr Opin Cell Biol 11:432-9). Interestingly, the PDZ domain found in many MAGUK proteins has been shown to interact with the intracellular domains of specific receptors. Thus, CARD-11 may function as a scaffolding protein to assemble a multi-protein **complex** at the intracellular domain of a receptor that signals the activation of NF-kB.

Detail Description Paragraph - DETX (99):

[0231] The ability of CARD-10 to induce NF-.kappa.B activity was evaluated by using a luciferase reporter gene assay. When CARD-10 was expressed in 293T cells, NF-.kappa.B activity was induced 90-fold as compared to empty vector, in a CARD-10 concentration-dependent manner (FIG. 25A). Induction of NF-.kappa.B activity was dependent on the **IKK complex,** since dominant-negative versions of **IKK**-.gamma. and **IKK**-.beta. blocked the ability of CARD-10 to induce the activation of NF-.kappa.B.

Detail Description Paragraph - DETX (102):

[0234] The finding that CARD-10 both binds to Bcl-10 and signals NF-.kappa.B activation through its N-terminal CARD domain suggests that CARD-10 functions as an upstream activator of Bcl-10. CARD-10 is one of four CARD proteins identified thus far that assemble together with Bcl-10 and signal the activation of NF-.kappa.B (Bertin et al. 2000 J. Biol. Chem. 275:41082). These molecules (CARD-10, CARD-9, CARD-11, and CARD-14) likely function to transduce distinct upstream stimuli to the activation of Bcl-10 and NF-.kappa.B. This subclass of CARD proteins are related in both sequence and structure. In addition to containing closely related N-terminal CARDs that interact specifically with Bcl-10, each molecule contains a coiled-coiled domain that could mediate self-association resulting in aggregation and activation of Bcl-10 in response to upstream signals. Bcl-10 might then engage and oligomerize IKK.gamma. resulting in the activation of the **IKK complex** and NF-.kappa.B (Poyet et al. 2000 J. Biol. Chem. 275:37966; Inohara et al. 2000 J. Biol. Chem. 275:27823). Thus, CARD-10 and the other Bcl-10 activators (e.g., CARD-9, CARD-11 and CARD-14) likely function in a manner analogous to Apaf-1 and CARD-4, molecules that induce oligomerization and activation of their respective downstream CARD-binding partners. CARD-10, CARD-11, and CARD-14 each contain a C-terminal PDZ/SH3/GUK domain, the presence of which suggests a role for these proteins in signal transduction by receptors at the plasma membrane. A recent study implicating Bcl-10 as a mediator of antigen receptor signaling in B and T cells suggests that CARD-10 and the other CARD/MAGUK family members might function to recruit Bcl-10 to receptor complexes. For example, signaling complexes at the plasma membrane (e.g., T

and B cell receptors) may recruit and activate the CARD/MAGUK proteins (CARD-10, CARD-11, and CARD-14) through their C-terminal PDZ/SH3/GUK domains. Bcl-10 might then engage and oligomerize IKK.gamma. resulting in the activation of the **IKK complex** and NF-.kappa.B.

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ABSTRACT:

The invention relates to the modulation of gene expression in a cell, also called gene control, in particular in relation to the treatment of a variety of diseases. The invention provides a method for modulating expression of a gene in a cell comprising providing said cell with a signalling molecule comprising a peptide or functional analogue thereof. Furthermore, the invention provides a method for identifying or obtaining a signalling molecule comprising a peptide or functional derivative or analogue thereof capable of modulating expression of a gene in a cell comprising providing said cell with a peptide or derivative or analogue thereof and determining the activity and/or nuclear translocation of a gene transcription factor.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (46):

[0045] NF-.kappa.B represents a group of structurally related and evolutionarily conserved gene transcription factors. So far, five mammalian NF-.kappa.B proteins named Rel (c-Rel), RelA (p65), RelB, NF-.kappa.B (p50 and its precursor p105), and NF-.kappa.B (p52 and it precursor p100) have been described. NF-.kappa.B proteins can exist as homo- or heterodimers, and although most NF-.kappa.B dimers are activators of transcription, the p50/p50 and p52/p52 homodimers often repress the transcription of their target genes. In Drosophila, three NF-.kappa.B homologs named Dorsal, Dif, and Relish have

been identified and characterized. Structurally, all NF-.kappa.B/Rel proteins share a highly conserved NH.sub.2-terminal Rel homology domain (RHD) that is responsible for DNA binding, dimerization, and association with inhibitory proteins known as I.kappa.BS. In resting cells, NF-.kappa.B/Rel dimers are bound to I.kappa.Bs and retained in an inactive form in the cytoplasm. Like NF-.kappa.B, IkBs are also members of a multigene family containing seven known mammalian members including I.kappa.B.alpha., I.kappa.B.beta., I.kappa.b.gamma., I.kappa.B.epsilon., Bcl-3, the precursor Rel-proteins, p100, and p105, and one Drosophila I.kappa.B named Cactus. The I.kappa.B family is characterized by the presence of multiple copies of ankyrin repeats, which are protein-protein interaction motifs that interact with NF-.kappa.B via the RHD. Upon appropriate stimulation, I.kappa.B is phosphorylated by I.kappa.B kinases (**IKKs**), polyubiquitinated by a ubiquitin ligase **complex,** and degraded by the 26S proteosome. Consequently, NF-.kappa.B is released and translocates into the nucleus to initiate gene expression.

Summary of Invention Paragraph - BSTX (61):

[0060] NF-kappaB/Rel proteins are a group of structurally related and evolutionarily conserved proteins (Rel). Well known are c-Rel, RelA (p65), RelB, NF-kappaB 1 (p50 and its precursor p105), and NF-kappaB2 (p52 and its precursor p100). Most NF-kappaB dimers are activators of transcription, p50/p50 and p52/p52 homodimers repress the transcription of their target genes. All NF-kappaB/Rel proteins share a highly conserved NH2-terminal Rel homology domain (RHD). RHD is responsible for DNA binding, dimerization, association with inhibitory proteins known as IkappaBs. In resting cells, NF-kappaB/Rel dimers are bound to IkappaBs and retained in an inactive form in the cytoplasm. IkappaBs are members of a multigene family (IkappaBalpha, IkappaBbeta, IkappaBgamma, IkappaBepsilon, Bcl-3, the precursor Rel-proteins, p100 and p105. Presence of multiple copies of ankyrin repeats to interact with NF-kappaB via the RHD (protein-protein interaction. Upon appropriate stimulation, IkappaB is phosphorylated by IkappaB Kinase (**IKKs**), polyubiquitinated by ubiquitin ligase **complex,** and degraded by the 26S proteosome. NF-kappaB is released and translocates into nucleus to initiate gene expression.

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INVENTOR-INFORMATION:

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ABSTRACT:

The present invention relates to substituted indazole derivatives, compositions comprising such, intermediates, methods of making substituted indazolel derivatives, and methods for treating cancer, inflammation, and inflammation-associated disorders, such as arthritis.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0004] NF-.kappa.B is a ubiquitous transcription factor that plays a prominent role in the activation of the immune system and in stress responses by regulating the transcription of many early, inducible genes including proinflammatory cytokines, adhesion molecules, growth factors, enzymes, and receptors (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16,

115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342). Specificity of gene expression is determined at a cellular level by a diverse array of external stimuli such as bacterial products including LPS, as well as cytokines, most importantly tumor necrosis factor-.alpha. (TNF.alpha.) and interleukin-.beta. (IL1.beta.). Through the synergistic interaction with other transcription factors, further specificity can be achieved while maintaining enormous potential to coordinately induce a large number of functionally related genes. NF-.kappa.B is composed of homo and heterodimers of the Rel protein family and is sequestered in an inactive form in the cytoplasm by members of the I.kappa.B family of inhibitory proteins (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342). I.kappa.Bs mask the nuclear localization signal on NF-.kappa.B, preventing nuclear translocation and hence DNA binding to the promoter regions of responsive genes. Stimulation of cells with an agonist that activates NF-.kappa.B leads to a series of biochemical signals, ultimately resulting in the phosphorylation, ubiquitinylation, and degradation of I.kappa.BS, thereby releasing NF-.kappa.B for nuclear translocation (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342). Recently, two I.kappa.B kinases (**IKK1** or IKK.alpha. and **IKK2** or IKK.beta.), which phosphorylate I.kappa.Bs and thereby initiate their degradation, have been cloned and characterized by a number of laboratories (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342). The catalytic subunits, **IKK1 and IKK2,** are similar structurally as well as enzymatically and exist as a heterodimer in a large protein **complex** referred to as the **IKK** signalsome (Regnier, C., Song, H., Gao, X., Goeddel, D., Cao, Z. and Rothe, M. (1997) Cell 90, 373-383; DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E. and Karin, M. (1997) Nature 388, 548-554; Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. and Roa, A. (1997) Science 278, 860-866; Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Woronicz, J. D., Gao, X., Cao, Z., Rothe, M. And Goeddel, D. V. (1997) Science 278, 866-869). A third protein, NEMO (IKK.gamma., IKKAP1), is a regulatory adapter protein necessary for **IKK** activation and kinase activity (Yamaoka, S., Courtois, G., Bessia, C., Whiteside, S. T., Weil, R., Agou, F., Kirk, H. E., Kay, R. J., and Ireal, A. (1998) Cell 93, 1231-1240; Rothwarf, D. M., Zandi, E., Natoli, G., Karin, M. (1998) Nature 395, 297; Mercurio, F., Murray, B. W., Shevchenko, A., Bennet, B. L., Young, D. B., Li, J. W., Pascual, G., Motiwala, A., Zhu, H., Mann, M and Manning, A. M. (1999) Mol. Cell. Biol 2, 1526-1538). **IKK1 and IKK2** are co-expressed in most human adult tissues as well as in different developmental stages of mouse embryos (Regnier, C., Song, H., Gao, X., Goeddel, D., Cao, Z. and Rothe, M. (1997) Cell 90, 373-383; DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E. and Karin, M. (1997) Nature 388, 548-554; Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. and Roa, A. (1997) Science 278, 860-866; Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Woronicz, J. D., Gao, X., Cao, Z., Rothe, M. and Goeddel, D. V. (1997) Science 278, 866-869; Hu, M. C. T., and Wang, Y. (1998) Gene 222, 31-40). This kinase **complex** appears to represent a critical,

common denominator in the activation of NF-.kappa.B in a number of signal transduction pathways stimulated by a variety of agonists including cytokines, such as TNF.alpha. and IL1.beta., microbial products such as LPS and viral proteins such as TAX, as well as phorbol esters, oxidizing agents and serine/tyrosine phosphatases (Ghosh S., May, M. J., and Kopp. E (1998) Annu. Rev. Immunol. 16, 115-260; Zandi, E., and Karin, M. (1999) Mol. Cell. Biol. 19, 4547-4551; Karin, M. (1999) J. Biol. Chem. 274, 27339-27342).

Summary of Invention Paragraph - BSTX (6):

[0005] **IKK1** (also termed IKK.alpha., Regnier, C., Song, H., Gao, X., Goeddel, D., Cao, Z. and Rothe, M. (1997) Cell 90, 373-383; DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E. and Karin, M. (1997) Nature 388, 548-554; Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. And Roa, A. (1997) Science 278, 860-866) was cloned simultaneously by standard biochemical purification of the I.kappa.B kinase activity from TNF.alpha. stimulated HeLa S3 cells and by its interaction with the MAP3K, NF-.kappa.B inducing kinase (NIK), in a yeast two-hybrid screen. **IKK1** was identified as the previously cloned serine-threonine kinase, CHUK (Connelly, M. and Marcu, K. (1995) Cell. Mol. Biol. Res. 41, 537-549). **IKK1** (also termed IKK.alpha.) is an 85 kDa, 745 amino acid protein that contains an N-terminal serine/threonine kinase catalytic domain, a leucine zipper-like amphipathic helix, and a C-terminal helix-loop-helix domain. **IKK2** (also termed IKK.beta.) was also cloned by standard biochemical purification, copurifying with **IKK1** from TNF.alpha. stimulated HeLa S3 cells as well as by being identified in the public database from an EST clone with sequence homology to **IKK1** (Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. and Roa, A. (1997) Science 278, 860-866; Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Woronicz, J. D., Gao, X., Cao, Z., Rothe, M. And Goeddel, D. V. (1997) Science 278, 866-869). **IKK2** is an 87 kDa, 756 amino acid protein with the same over all topology as **IKK1** except for the addition of an 11 amino acid extension at the C-terminus. **IKK1 and IKK2** are 52% identical overall with 65% identity in the kinase domain and 44% identity in the protein interaction domains in the C-terminus. Data obtained using transient mammalian expression analysis, by in vitro translation experiments and by coexpression in a baculoviral system reveals that **IKK1 and IKK2** associate preferentially as a heterodimer through their leucine zipper motifs. Although homodimers have also been described in these systems, the heterodimer is thought to be the physiologic form of the kinase in mammalian cells (Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Li, J., Peet, G. W., Pullen, S. S., Schembri-King, J., Warren, T. C., Marcu, K. B., Kehry, M. R., Barton, R. and Jakes, S. (1998) J. Biol. Chem. 273, 30736-30741). Finally, NEMO (also termed IKK.gamma.) contains three .alpha.-helical regions including a leucine zipper, interacts preferentially with **IKK2** and is required for activation of the heterodimeric kinase **complex** perhaps by bringing other proteins into the signalsome **complex** (Yamaoka, S., Courtois, G., Bessia, C., Whiteside, S. T., Weil, R., Agou, F., Kirk, H. E., Kay, R. J., and Ireal, A. (1998) Cell 93, 1231-1240; Rothwarf, D. M., Zandi, E., Natoli, G., Karin, M. (1998) Nature 395, 297; Mercurio, F., Murray, B. W., Shevchenko, A., Bennet, B. L., Young, D. B., Li, J. W., Pascual, G., Motiwala, A., Zhu, H., Mann, M and

Manning, A. M. (1999) Mol. Cell. Biol. 2, 1526-1538).

Summary of Invention Paragraph - BSTX (8):

[0007] **IKK2** demonstrates a more potent kinase activity compared to **IKK1** using I.kappa.B.alpha. or I.kappa.B.beta. as a substrate (Mercurio, F., Zhu, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J. W., Young, D. B., Barbosa, M., Mann, M., Manning, A. and Roa, A. (1997) Science 278, 860-866; Zandi, E. Rothwarf, D. M., Delhase, M., Hayadawa, M and Karin, M. (1997) Cell 91, 243-252; Woronicz, J. D., Gao, X., Cao, Z., Rothe, M. and Goeddel, D. V. (1997) Science 278, 866-869; Dehase, M., Hayakawa, M., Chen, Y., and Karin, M. (1999) Science 284, 309-313). Mutations of the phospho-acceptor serine residues within the MAPKK activation loop alters **IKK2** kinase activity; the serine to alanine substitutions result in decreased kinase activity whereas the serine to glutamic acid substitutions result in a constitutively active kinase. Similar alanine mutations in **IKK1** do not result in a decreased stimulation of total **IKK** activity in response to TNF.alpha. or IL1.beta. (Dehase, M., Hayakawa, M., Chen, Y., and Karin, M. (1999) Science 284, 309-313). **IKK2** being the dominant kinase activity within the **IKK complex** is further supported by the analysis of fibroblasts from mice deficient in **IKK1 or IKK2**. Fibroblasts lacking **IKK1** retain full **IKK** activity in response to cytokines and could activate NF-.kappa.B. In contrast, fibroblasts lacking **IKK2** do not exhibit **IKK** activity when stimulated with cytokines nor do they activate NF-.kappa.B. Furthermore, the phenotypes of each **IKK** knock out is unique with **IKK1** deficiency resulting in skin and skeletal defects and **IKK2** knock out being embryonic lethal due to hepatocyte apoptosis (Li, Q., Antwerp, D. V., Mercurio, F., Lee, K., and Verma, I. M. (1999) Science 284, 321-325; Takeda, K., Tekeuchi, O., Tsujimura, T., Itami, S., Adachi, O., Kawai, T., Sanjo, H., Yoshikawa, K., Terada, N, and Akira, S. (1999) Science 284, 313-316; Hu, Y., Baud, V., Delhase, M., Zhang, P., Deerinck, T., Ellisman, M., Johnson, R., and Karin, M. (1999) Science 284, 315-320; Li, Q., Lu, Q., Hwang, J. Y., Buscher, D., Lee, K., Izpisua-Belmonte, J. C., and Verma, I. M. (1999) Gene and Development 13, 1322-1328; Tanaka, M., Fuentes, M. E., Yamaguchi, K., Durnin, M. H., Dalrymple, S. A., Hardy, K. L., and Goeddel, D. V. (1999) Immunity 10, 421-429).

Detail Description Paragraph - DETX (157):

[0195] SF9 cells paste containing rhIKKs were centrifuged (100,000.times.g, 10 min) to remove debris. rhIKKs were immunoprecipitated (100 .mu.g of cell paste) from the cell supernatant using 3 .mu.g of anti-NEMO antibody (FL-419), followed by coupling to protein A sepharose beads. rhIKKs were also immunoprecipitated from affinity chromatography purified protein preparations (1 .mu.g) using anti-FLAG, anti-His or anti-NEMO antibodies (1-4 .mu.g) followed by protein A sepharose coupling. The native, human **IKK complex** was immunoprecipitated from THP-1 cell homogenates (300 .mu.g/condition) using the anti-NEMO antibody. Immune complexes were pelleted and washed 3 times with 1 ml cold lysis buffer. Immunoprecipitated rhIKKs were chromatographed by SDS-PAGE (8% Tris-glycine) and transferred to nitrocellulose membranes (Novex) and detected by chemiluminescense (SuperSignal) using specific anti-**IKK** antibodies (**IKK2** H-470, **IKK1** H-744). Native **IKK2**, I.kappa.B.alpha. and NEMO proteins from cytosolic lysates (20-80 .mu.g) were separated by SDS-PAGE and

visualized by chemiluminescense using specific antibodies.

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TITLE: Novel molecules of the card-related protein family and uses thereof

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INVENTOR-INFORMATION:

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ABSTRACT:

Novel CARD-9, CARD-10, or CARD-11 polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated CARD-9, CARD-10, or CARD-11 proteins, the invention further provides CARD-9, CARD-10, or CARD-1l, fusion proteins, antigenic peptides and anti-CARD-9, CARD-10, or CARD-11 antibodies. The invention also provides CARD-9, CARD-10, or CARD-11 nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced

and non-human transgenic animals in which a CARD-9, CARD-10 or CARD-11 gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. application Ser. No. 09/728,260, filed Dec. 1, 2000, which is a continuation-in-part of U.S. application Ser. No. 09/685,791, filed Oct. 10, 2000, which is a continuation-in-part of U.S. application Ser. No. 09/513,904, filed Feb. 25, 2000, which is a continuation-in-part of application Ser. No. 09/507,533, filed Feb. 18, 2000, which claimed priority from provisional application Serial No. 60/168,780, filed Dec. 3, 1999. The content of each of these applications is herein incorporated by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):

[0009] Nuclear factor-.kappa.B (NF-.kappa.B) is a transcription factor that is expressed in many cell types and activates genes that have NF-.kappa.B sites in their promoters. Molecules that regulate NF-.kappa.B activation play a critical role in both apoptosis and in the stress-response of cells. With respect to stress-reponse, NF-.kappa.B activates genes that control immune defense mechanisms and inflammation. The CARD-containing proteins RICK, CARD-4 and Bcl-10 also induce activation of the NF-.kappa.B transcription factor suggesting that CARD/CARD signaling complexes regulate activation of the **IKK complex** (Inohara et al. 1998 Proc. Natl. Acad Sci. USA 273:12296; Bertin et al. 1999 J. Biol. Chem. 224:12955; Willis et al. 1999 Cell 96: 33). In unstimulated cells, NF-.kappa.B is found sequestered in the cytoplasm through interactions with inhibitory I.kappa.B proteins. Inhibition is relieved by the phosphorylation and proteosomal degradation of I.kappa.B proteins by proinflammatory cytokines. Phosphorylation is mediated by the **IKK complex** which is comprised of at least three major proteins: two kinases designated IKK.alpha. and IK.beta. that directly phosphorylate the I.kappa.B inhibitory proteins, and a noncatalytic subunit called IKK.gamma. that functions to link the **IKKs** to upstream regulatory molecules (Zhang et al., 2000). Recently, RICK has been found to function as upstream regulatory molecules of the **IKK complex** (Inohara et al. 2000 J. Biol. Chem. 275:27823). RICK interacts directly with IKK.gamma. suggesting that it functions as signaling adaptor between the **IKK complex** and an upstream CARD-containing NF-.kappa.B activator. Indeed, CARD-4 forms a CARD/CARD signaling **complex** with RICK that induces activation of the **IKK complex** and the subsequent release of NF-.kappa.B (Bertin et al. 1999 J. Biol. Chem. 224:12955; Inohara et al. 1999 J. Biol. Chem. 274:14566; Inohara et al. 2000 J. Biol. Chem. 275:27823).

Detail Description Paragraph - DETX (78):

[0207] These results, taken with the finding of a direct interaction between CARD-9 and Bcl-10 suggest that CARD-9 is a specific regulator of Bcl-10 function. CARD-9 could play a role as an upstream signaling molecule that

recruits Bcl-10 through CARD/CARD interactions. The resulting signaling **complex** may interact directly or indirectly with components of the **IKK complex** resulting in its activation, e.g., through oligomerization of IKK.gamma.. Indeed the data described above data shows that both CARD-9 and Bcl-10 form large oligomeric complexes (filaments) when overexpressed in mammalian cells. Furthermore, enforced oligomerization of the C-terminus of Bcl-10/CLAP is thought to induce NF-.kappa.B activation, suggesting that the CARD domain of Bcl-10 functions as an oligomerization domain that transduces the activation signal to the **IKK complex** through its C-terminal domain. The ability of CARD-9 to form a **complex** with Bcl-10 via CARD/CARD interactions supports the idea that Bcl-10 functions as an adaptor between the effector **IKK complex** and the proximal signaling complexes that interact with CARD-9. Signaling molecules upstream of CARD-9 are predicted to transduce their signals to Bcl-10 through direct interactions with the C-terminal coiled-coil domain of CARD-9. Taken together, these results identify CARD-9 as an important mediator of NF-.kappa.B signaling through Bcl-10.

Detail Description Paragraph - DETX (81):

[0210] These studies showed that when CARD-11 is expressed in 293T cells, NF-kB activity is induced 20- to 40-fold compared to empty vector (FIG. 20A). NF-kB signaling occurred through the **IKK complex** since dominant-negative versions of **IKK-g and IKK-b** blocked the ability of CARD-11 to induce NF-kB activity (data not shown). To determine the role of individual domains in NF-kB signaling, a series of N- and C-terminal truncation mutants of CARD-11 were constructed (FIG. 20B). The N-terminal CARD of CARD-11 was essential for NF-kB signaling since deletion of this domain eliminated the induction of NF-kB activity (FIG. 20C). Immunoblot analysis revealed that the mutant proteins were expressed at levels similar to wt protein indicating that loss of function was not due to reduced levels of expression. In contrast, the C-terminal PDZ, SH3 and GUK domains were not required for NF-kB signaling since deletion of these domains had no effect on the ability of CARD-11 to induce NF-kB activity. However, a CARD-11 mutant lacking its C-terminal PDZ, SH3 and GUK domains induced NF-kB activity to levels 4- to 5-fold greater than that obtained with wt protein (FIG. 20C). Thus, the C-terminal domains may function to negatively regulate induction of NF-kB signaling by CARD-11.

Detail Description Paragraph - DETX (90):

[0219] CARD-11 is a specific regulator of Bcl-10 function. The finding that CARD-11 binds to Bcl-10 through a CARD/CARD interaction suggests that this molecule functions as upstream activator of Bcl-10. As discussed above, CARD-9 also binds to the CARD activation domain of Bcl-10 and signals NF-kB activation. Thus, CARD11 and CARD-9 constitute a subclass of CARD proteins that may function to transduce upstream stimuli to the activation of Bcl-10 and NF-kB. In response to upstream signals, the coiled-coil domains could mediate self-association of CARD-11 resulting in the aggregation and activation of Bcl-10. Bcl-10 might then engage and oligomerize **IKKg** resulting in the activation of the **IKK complex** and NF-kB (Inohara et al. 1999 J. Biol. Chem. 274:14566; Poyet et al., 1999). Thus, CARD-11 could function in a manner analogous to Apaf-1 and CARD-4 that function as upstream regulators to induce oligomerization and activation of their respective downstream CARD binding partners. The data showing that CARD-11 induces the phosphorylation of Bcl-10

suggests that signal transduction may involve the participation of a serine/threonine kinase. The C-terminal PDZ/SH3/GUK domains of CARD-11 may function in an analogous manner to the C-terminal LRR domain of CARD-4 and the WD-40 domain of Apaf-1 to regulate protein activation by upstream signals. PDZ/SH3/GUK domains identify MAGUK family members, a class of proteins that associate with the plasma membrane (Fanning and Anderson, 1999 Curr Opin Cell Biol 11:432-9). Interestingly, the PDZ domain found in many MAGUK proteins has been shown to interact with the intracellular domains of specific receptors. Thus, CARD-11 may function as a scaffolding protein to assemble a multi-protein **complex** at the intracellular domain of a receptor that signals the activation of NF-kB.

Detail Description Paragraph - DETX (99):

[0228] The ability of CARD-10 to induce NF-.kappa.B activity was evaluated by using a luciferase reporter gene assay. When CARD-10 was expressed in 293T cells, NF-.kappa.B activity was induced 90-fold as compared to empty vector, in a CARD-10 concentration-dependent manner (FIG. 25A). Induction of NF-.kappa.B activity was dependent on the **IKK complex,** since dominant-negative versions of **IKK**-.gamma. and **IKK**-.beta. blocked the ability of CARD-10 to induce the activation of NF-.kappa.B.

Detail Description Paragraph - DETX (102):

[0231] The finding that CARD-10 both binds to Bcl-10 and signals NF-.kappa.B activation through its N-terminal CARD domain suggests that CARD-10 functions as an upstream activator of Bcl-10. CARD-10 is one of four CARD proteins identified thus far that assemble together with Bcl-10 and signal the activation of NF-.kappa.B (Bertin et al. 2000 J. Biol. Chem. 275:41082). These molecules (CARD-10, CARD-9, CARD-11, and CARD-14) likely function to transduce distinct upstream stimuli to the activation of Bcl-10 and NF-.kappa.B. This subclass of CARD proteins are related in both sequence and structure. In addition to containing closely related N-terminal CARDs that interact specifically with Bcl-10, each molecule contains a coiled-coiled domain that could mediate self-association resulting in aggregation and activation of Bcl-10 in response to upstream signals. Bcl-10 might then engage and oligomerize IKK.gamma. resulting in the activation of the **IKK complex** and NF-.kappa.B (Poyet et al. 2000 J. Biol. Chem. 275:37966; Inohara et al. 2000 J. Biol. Chem. 275:27823). Thus, CARD-10 and the other Bcl-10 activators (e.g., CARD-9, CARD-11 and CARD-14) likely function in a manner analogous to Apaf-1 and CARD-4, molecules that induce oligomerization and activation of their respective downstream CARD-binding partners. CARD-10, CARD-11, and CARD-14 each contain a C-terminal PDZ/SH3/GUK domain, the presence of which suggests a role for these proteins in signal transduction by receptors at the plasma membrane. A recent study implicating Bcl-10 as a mediator of antigen receptor signaling in B and T cells suggests that CARD-10 and the other CARD/MAGUK family members might function to recruit Bcl-10 to receptor complexes. For example, signaling complexes at the plasma membrane (e.g., T and B cell receptors) may recruit and activate the CARD/MAGUK proteins (CARD-10, CARD-11, and CARD-14) through their C-terminal PDZ/SH3/GUK domains. Bcl-10 might then engage and oligomerize IKK.gamma. resulting in the activation of the **IKK complex** and NF-.kappa.B.

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INVENTOR-INFORMATION:

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ABSTRACT:

Provided are methods and compositions for assaying for ubiquitin agents that are enzymatic components of ubiquitin-mediated proteolysis and, more particularly, methods and compositions for assaying for agents that modulate the activity of such ubiquitin agents.

[0001] This application is a continuation-in-part application of U.S. patent application Ser. No. 09/826,312, filed Apr. 3, 2001 (pending) which is a continuation-in-part application U.S. patent application Ser. No. 09/542,497, filed Apr. 3, 2000 (pending).

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (10):

[0009] Two major classes of E3 ubiquitin ligating agents are known: the HECT (homologous to E6-AP carboxy terminus) domain E3 ligating agents; and the RING finger domain E3 ligating agents. E6AP is the prototype for the HECT domain subclass of E3 ligating agents and is a multi-subunit **complex** that functions as a ubiquitin ligating agent for the tumor suppressor p53 which is activated by papillomavirus in cervical cancer (Huang et al. (1999) Science 286:1321-1326). Members of this class are homologous to the carboxyl terminus of E6AP and utilize a Cys active site to form a thiolester bond with ubiquitin, analogous to the E1 activating agents and E2 conjugating agents. However, in contrast, the members of the RING finger domain class of E3 ligating agents are thought to interact with an ubiquitin--conjugated-E2 intermediate to activate the **complex** for the transfer of ubiquitin to an acceptor. Examples of the RING domain class of E3 ligating agents are TRAF6, involved in **IKK** activation; Cbl, which targets insulin and EGF; Sina/Siah, which targets DCC; Itchy, which is involved in haematopoesis (B, T and mast cells); IAP, involved with inhibitors of apoptosis; and Mdm2 which is involved in the regulation of p53.

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TITLE: Antisense modulation of inhibitor-kappa B-R expression

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INVENTOR-INFORMATION:

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ABSTRACT:

Antisense compounds, compositions and methods are provided for modulating the expression of inhibitor-kappa B-R. The compositions comprise antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding inhibitor-kappa B-R. Methods of using these compounds for modulation of inhibitor-kappa B-R expression and for treatment of diseases associated with expression of inhibitor-kappa B-R are provided.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0003] The activity of NF-.kappa.B is regulated by inhibitor .kappa.B proteins (I.kappa.Bs). I.kappa.Bs retain NF-.kappa.B in an inactive state in the cytoplasm of non-stimulated cells, until an immune, inflammatory, apoptotic response is required. Diverse stimuli, such as cytokines, viral double-stranded RNA and viral transactivator proteins, bacterial lipopolysaccharides (LPS), or ionizing radiation trigger a rapid first line of cellular defense in which a multisubunit I.kappa.B kinase (**IKK) complex** phosphorylates I.kappa.B, marking I.kappa.B for ubiquitination and proteolytic degradation and freeing NF-.kappa.B to translocate to its nuclear site of action (Karin and Delhase, Semin. Immunol., 2000, 12, 85-98).

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TITLE: Antisense modulation of inhibitor-kappa B kinase-gamma expression

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APPL-NO: 09/ 972607

DATE FILED: October 6, 2001

US-CL-CURRENT: 514/44, 435/375 , 536/23.2

ABSTRACT:

Antisense compounds, compositions and methods are provided for modulating the expression of inhibitor-kappa B kinase-gamma. The compositions comprise antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding inhibitor-kappa B kinase-gamma. Methods of using these compounds for modulation of inhibitor-kappa B kinase-gamma expression and for treatment of diseases associated with expression of inhibitor-kappa B kinase-gamma are provided.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0003] The activity of NF-.kappa.B is regulated by inhibitor .kappa.B proteins (I.kappa.Bs). I.kappa.Bs retain NF-.kappa.B in an inactive state in the cytoplasm of non-stimulated cells, until an immune, inflammatory, apoptotic response is required. Diverse stimuli, such as cytokines, viral double-stranded RNA and viral transactivator proteins, bacterial lipopolysaccharides (LPS), or ionizing radiation, trigger a rapid first line of cellular defense in which a multisubunit I.kappa.B kinase (**IKK) complex** phosphorylates I.kappa.B, marking I.kappa.B for ubiquitination and proteolytic degradation and freeing NF-.kappa.B to translocate to its nuclear site of action (Karin and Delhase, Semin. Immunol., 2000, 12, 85-98; Zandi and Karin, Mol. Cell Biol., 1999, 19, 4547-4551).

Summary of Invention Paragraph - BSTX (6):

[0004] The 900 kDa protein kinase **complex IKK** is considered the master regulator of NF-.kappa.B-mediated innate immune and inflammatory responses. This **complex** was defined based on its ability to catalyze the phosphorylation of two N-terminal regulatory serines in I.kappa.B proteins, and its rapid activation in response to cell stimulation by tumor necrosis factor alpha (TNF.alpha.), interleukin-1 (IL-1) or LPS. The **IKK complex** comprises three subunits: the catalytic subunits inhibitor kappa-B kinase-alpha (IKK.alpha.) and inhibitor kappa-B kinase-beta (IKK.beta.), and the regulatory subunit inhibitor kappa-B kinase-gamma (IKK.gamma.; also known as I.kappa.B kinase gamma subunit, **IKK**-gamma, **IKK-3**, NF-kappa-B essential modulator, NEMO, FIP3, Fip3p, IP2, IKKAP1, **IKK**-associated protein 1, inhibitor of kappa light polypeptide gene enhancer in B-cells kinase gamma). Fully active **IKK** complexes purified from mammalian cells consist of IKK.alpha.:IKK.beta. heterodimers associated with one or several inhibitor kappa-B kinase-gamma molecules, and in the absence of inhibitor kappa-B kinase-gamma, no activation of **IKK** or NF-.kappa.B can occur (Karin and Delhase, Semin. Immunol., 2000, 12, 85-98; Zandi and Karin, Mol. Cell Biol., 1999, 19, 4547-4551).

Summary of Invention Paragraph - BSTX (7):

[0005] Using a monoclonal antibody to the IKK.alpha. subunit, the large, cytokine-responsive **IKK complex** was purified to homogeneity from human HeLa and Jurkat cell lines and found to contain equimolar amounts of IKK.alpha. and IKK.beta., as well as two additional polypeptides of 50 and 52 kDa. Microsequencing and molecular cloning revealed that these polypeptides are differentially processed forms of a third component of **IKK**, inhibitor kappa-B kinase-gamma. Secondary structural analysis indicated that inhibitor kappa-B kinase-gamma is composed of several coiled-coil motifs, and includes a leucine-zipper and a Zn-finger at its C-terminus (Rothwarf et al., Nature, 1998, 395, 297-300).

Summary of Invention Paragraph - BSTX (8):

[0006] Human inhibitor kappa-B kinase-gamma was cloned independently using a mutant cell line, 5R, a cellular flat variant of Rat-1 fibroblasts transformed by the Tax transactivator oncoprotein of human T-cell leukemia virus type 1 (HTLV-1). Tax causes persistent activation of NF-.kappa.B due to constitutive activation of **IKK**, and is associated with cellular transformation, but 5R cells carry a recessive mutation abolishing the Tax-induced activation of NF-.kappa.B. In a genetic complementation approach, inhibitor kappa-B kinase-gamma was identified based on its ability to restore Tax-mediated NF-.kappa.B activation to 5R mutant cells, and was found to be a critical component of the **IKK complex**, involved in inducible I.kappa.B kinase activity (Yamaoka et al., Cell, 1998, 93, 1231-1240). It was later shown that Tax binds to neither IKK.alpha. or IKK.beta. but complexes directly with inhibitor kappa-B kinase-gamma, correlating with Tax-induced phosphorylation of NF-.kappa.B activation (Jin et al., J. Biol. Chem., 1999, 274, 17402-17405), and that inhibitor kappa-B kinase-gamma enables Tax to dock with the IKK.beta. catalytic subunit of the **IKK complex**, acting as an adapter protein that directs stable formation of pathologic Tax-**IKK** complexes in virally infected T-cells (Chu et al., J. Biol. Chem., 1999, 274, 15297-15300).

Summary of Invention Paragraph - BSTX (14):

[0012] A cell-permeable peptide representing a carboxyl-terminal segment of IKK.alpha. or IKK.beta. known as the NEMO-binding domain (NBD) blocked association of inhibitor kappa-B kinase-gamma with the **IKK complex,** inhibited cytokine-induced NF-.kappa.B-=dependent gene expression, and ameliorated the inflammatory response in two experimental mouse models of acute inflammation, suggesting that the NBD is a target for drug development (May et al., Science, 2000, 289, 1550-1554).

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TITLE: Assays for identifying ubiquitin agents and for identifying agents that modify the activity of ubiquitin agents

PUBLICATION-DATE: June 5, 2003

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/7.1, 435/23

ABSTRACT:

Provided are methods and compositions for assaying for ubiquitin agents that are enzymatic components of ubiquitin-mediated proteolysis and, more particularly, methods and compositions for assaying for agents that modulate the activity of such ubiquitin agents.

[0001] This application is a continuation-in-part application of U.S. patent application Ser. No. 09/826,312, filed Apr. 3, 2001 (pending) which is a continuation-in-part application U.S. patent application Ser. No. 09/542,487, filed Apr. 3, 2000 (pending).

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (10):

[0009] Two major classes of E3 ubiquitin ligating agents are known: the HECT (homologous to E6-AP carboxy terminus) domain E3 ligating agents; and the RING finger domain E3 ligating agents. E6AP is the prototype for the HECT domain subclass of E3 ligating agents and is a multi-subunit **complex** that functions as a ubiquitin ligating agent for the tumor suppressor p53 which is activated by papillomavirus in cervical cancer (Huang et al. (1999) Science 286:1321-1326). Members of this class are homologous to the carboxyl terminus of E6AP and utilize a Cys active site to form a thiolester bond with ubiquitin, analogous to the E1 activating agents and E2 conjugating agents. However, in contrast, the members of the RING finger domain class of E3 ligating agents are thought to interact with an ubiquitin-conjugated-E2 intermediate to activate the **complex** for the transfer of ubiquitin to an acceptor. Examples of the RING domain class of E3 ligating agents are TRAF6, involved in **IKK** activation; Cbl, which targets insulin and EGF; Sina/Siah, which targets DCC; Itchy, which is involved in haematopoesis (B, T and mast cells); IAP, involved with inhibitors of apoptosis; and Mdm2 which is involved in the regulation of p53.

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DOCUMENT-IDENTIFIER: US 20030100026 A1

TITLE: Stimulus-inducible protein kinase complex and methods of use therefor

PUBLICATION-DATE: May 29, 2003

INVENTOR-INFORMATION:

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ABSTRACT:

Compositions and methods are provided for treating NF-.kappa.B-related conditions. In particular, the invention provides a stimulus-inducible IKK signalsome, and components and variants thereof. An IKK signalsome or component thereof may be used, for example, to identify antibodies and other modulating agents that inhibit or activate signal transduction via the

NF-.kappa.B cascade. IKK signalsome, components thereof and/or modulating agents may also be used for the treatment of diseases associated with NF-.kappa.B activation.

CROSS-REFERENCE TO PRIOR APPLICATION

[0001] This is a divisional application of Ser. No. 08/910,820, filed Aug. 17, 1997 which is a continuation-in-part of U.S. patent application Ser. No. 08/697,393, filed Aug. 26, 1996.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (11):
[0033] FIGS. 8A-8C are autoradiograms depicting the results of immunoblot analyses. In FIG. 8A, the upper panel presents a time course for the induction of signalsome activity. Anti MKP-1 immune precipitates from extracts of HeLa S3 cells stimulated with TNF.alpha. (20 ng/ml) for the indicated times, were assayed for **IKK** signalsome activity by standard immune **complex** kinase assays. 4 .mu.g of either GST I.kappa.B.alpha. 1-54 WT (wildtype) or the GST I.kappa.B.alpha. 1-54 S32/36 to T mutant (S>T) were used as the substrates. In the lower panel, HeLa cell extracts prepared as described in the upper panel were examined by western blot analysis for I.kappa.B.alpha. degradation. I.kappa.B.alpha. supershifting phosphorylation can be seen after 3 and 5 minutes of stimulation followed by the disappearance of I.kappa.B.alpha..

Brief Description of Drawings Paragraph - DRTX (13):
[0035] FIG. 8C illustrates the ability of **IKK** signalsome to specifically phosphorylate serines 32 and 36 of the I.kappa.B.alpha. holoprotein in the context of a RelA:I.kappa.B.alpha. **complex**. Anti-MKP-1 immunoprecipitates from cell extracts of HeLa S3 cells stimulated with TNF.alpha. (20 ng/ml, 7 min) were examined for their ability to phosphorylate baculoviral expressed RelA:I.kappa.B.alpha. **complex** containing either the I.kappa.B.alpha. WT (lane 3) or I.kappa.B.alpha. S32/36 to A mutant (lane 4) holoprotein. The specific substrates used are indicated on the top. Positions of the phosphorylated substrates are indicated by arrows to the left of the panel.

Brief Description of Drawings Paragraph - DRTX (16):
[0038] FIG. 10 is an autoradiogram showing the results of a western blot analysis of the level of ubiquitin within a stimulus-inducible IkB kinase **complex**. Lane 1 shows the detection of 100 ng ubiquitin, Lane 2 shows 10 ng ubiquitin and Lane 3 shows 3.4 .mu.g of **IKK** signalsome purified through the phenyl superose step (sufficient quantities for 10 kinase reactions). The position of ubiquitin is shown by the arrow on the left.

Detail Description Paragraph - DETX (2):
[0047] As noted above, the present invention is generally directed to compositions and methods for modulating (i.e., stimulating or inhibiting) signal transduction leading to NF-.kappa.B activation. In particular, the

present invention is directed to compositions comprising an I.kappa.B kinase (**IKK**) signalsome (also referred to herein as a "stimulus-inducible I.kappa.B kinase **complex**" or "I.kappa.B kinase **complex**") that is capable of stimulus-dependent phosphorylation of I.kappa.B.alpha. and I.kappa.B.beta. on the two N-terminal serine residues critical for the subsequent ubiquitination and degradation in vivo. Such stimulus-dependent phosphorylation may be achieved without the addition of exogenous cofactors. In particular, an **IKK** signalsome specifically phosphorylates I.kappa.B.alpha. (SEQ ID NO:1) at residues S32 and S36 and phosphorylates I.kappa.B.beta. (SEQ ID NO:2) at residues S19 and S23. The present invention also encompasses compositions that contain one or more components of such an **IKK** signalsome, or variants of such components. Preferred components, referred to herein as "**IKK** signalsome kinases" "I.kappa.B kinases" or **IKKs**) are kinases that, when incorporated into an **IKK** signalsome, are capable of phosphorylating I.kappa.B.alpha. at S32 and S36. Particularly preferred components are **IKK-1** (SEQ ID NO:10) and **IKK-2** (SEQ ID NO:9).

Detail Description Paragraph - DETX (4):

[0049] An **IKK** signalsome has several distinctive properties. Such a **complex** is stable (i.e., its components remain associated following purification as described herein) and has a high-molecular weight (about 500-700 kD, as determined by gel filtration chromatography). As shown in FIGS. 3(A and B) and 4(A and B), I.kappa.B kinase activity of an **IKK** signalsome is "stimulus-inducible" in that it is stimulated by TNF.alpha. (i.e., treatment of cells with TNF.alpha. results in increased I.kappa.B kinase activity and I.kappa.B degradation) and/or by one or more other inducers of NF-.kappa.B, such as IL-1, LPS, TPA, UV irradiation, antigens, viral proteins and stress-inducing agents. The kinetics of stimulation by TNF.alpha. correspond to those found in vivo. I.kappa.B kinase activity of an **IKK** signalsome is also specific for S32 and S36 of I.kappa.B.alpha.. As shown in FIGS. 5(A and B) and 6(A and B), an **IKK** signalsome is capable of phosphorylating a polypeptide having the N-terminal sequence of I.kappa.B.alpha. (GST-I.kappa.B.alpha.1-54; SEQ ID NO:3), but such phosphorylation cannot be detected in an I.kappa.B.alpha. derivative containing threonine substitutions at positions 32 and 36. In addition, I.kappa.B kinase activity is strongly inhibited by a doubly phosphorylated I.kappa.B.alpha. peptide (i.e., phosphorylated at S32 and S36), but not by an unrelated c-fos phosphopeptide that contains a single phosphothreonine. A further characteristic of an **IKK** signalsome is its ability to phosphorylate a substrate in vitro in a standard kinase buffer, without the addition of exogenous cofactors. Free ubiquitin is not detectable in preparations of **IKK** signalsome (see FIG. 10), even at very long exposures. Accordingly an **IKK** signalsome differs from the ubiquitin-dependent I.kappa.B.alpha. kinase activity described by Chen et al., Cell 84:853-62, 1996.

Detail Description Paragraph - DETX (5):

[0050] An **IKK** signalsome may be immunoprecipitated by antibodies raised against MKP-1 (MAP kinase phosphatase-1; Santa Cruz Biotechnology, Inc., Santa Cruz, Calif. #SC-1102), and its activity detected using an in vitro I.kappa.B.alpha. kinase assay. However, as discussed further below, MKP-1

does not appear to be a component of I.kappa.B kinase **compl x**. The substrate specificity of the immunoprecipitated **IKK** signalsome is maintained (i.e., there is strong phosphorylation of wildtype GST-I.kappa.B.alpha. 1-54 (SEQ ID NO:3) and GST-I.kappa.B.beta. 1-44 (SEQ ID NO:4), and substantially no detectable phosphorylation of GST-I.kappa.B.alpha. 1-54 in which serines 32 and 36 are replaced by threonines (GST-I.kappa.B.alpha. S32/36 to T; SEQ ID NO:5) or GST-I.kappa.B.beta. 1-44 in which serines 19 and 23 are replaced by alanines (GST-I.kappa.B.beta. 1-44 S19/23 to A; SEQ ID NO:6)).

Detail Description Paragraph - DETX (8):

[0053] Throughout the fractionation, an in vitro kinase assay may be used to monitor the I.kappa.B kinase activity of the **IKK** signalsome. In such an assay, the ability of a fraction to phosphorylate an appropriate substrate (such as I.kappa.B.alpha. (SEQ ID NO:1) or a derivative or variant thereof) is evaluated by any of a variety of means that will be apparent to those of ordinary skill in the art. For example, a substrate may be combined with a chromatographic fraction in a protein kinase buffer containing .sup.32P .gamma.-ATP, phosphatase inhibitors and protease inhibitors. The mixture may be incubated for 30 minutes at 30.degree. C. The reaction may then be stopped by the addition of SDS sample buffer and analyzed using SDS-PAGE with subsequent autoradiography. Suitable substrates include full length I.kappa.B.alpha. (SEQ ID NO: 1), polypeptides comprising the N-terminal 54 amino acids of I.kappa.B.alpha., full length I.kappa.B.beta. (SEQ ID NO:2) and polypeptides comprising the N-terminal 44 amino acids of I.kappa.B.beta.. Any of these substrates may be used with or without an N-terminal tag. One suitable substrate is a protein containing residues 1-54 of I.kappa.B.alpha. and an N-terminal GST tag (referred to herein as GST-I.kappa.B.alpha. 1-54; SEQ ID NO:3). To evaluate the specificity of an I.kappa.B kinase **complex,** I.kappa.B.alpha. mutants containing threonine or alanine residues at positions 32 and 36, and/or other modifications, may be employed.

Detail Description Paragraph - DETX (9):

[0054] Alternatively, an **IKK** signalsome may be prepared from its components which are also encompassed by the present invention. Such components may be produced using well known recombinant techniques, as described in greater detail below. Components of an **IKK** signalsome may be native, or may be variants of a native component (i.e., a component sequence may differ from the native sequence in one or more substitutions and/or modifications, provided that the ability of a **complex** comprising the component variant to specifically phosphorylate I.kappa.B.alpha. is not substantially diminished). Substitutions and/or modifications may generally be made in non-critical and/or critical regions of the native protein. Variants may generally comprise residues of L-amino acids, D-amino acids, or any combination thereof. Amino acids may be naturally-occuring or may be non-natural, provided that at least one amino group and at least one carboxyl group are present in the molecule; .alpha.- and .beta.-amino acids are generally preferred. A variant may also contain one or more rare amino acids (such as 4-hydroxyproline or hydroxylysine), organic acids or amides and/or derivatives of common amino acids, such as amino acids having the C-terminal carboxylate esterified (e.g., benzyl, methyl or ethyl ester) or amidated and/or having modifications of the N-terminal amino group (e.g., acetylation or alkoxycarbonylation), with or

without any of a wide variety of side-chain modifications and/or substitutions (e.g., methylation, benzylation, t-butylation, tosylation, alkoxycarbonylation, and the like). Component variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids that have minimal influence on the activity of the polypeptide. In particular, variants may contain additional amino acid sequences at the amino and/or carboxy termini. Such sequences may be used, for example, to facilitate purification or detection of the component polypeptide. In general, the effect of one or more substitutions and/or modifications may be evaluated using the representative assays provided herein.

Detail Description Paragraph - DETX (11):

[0056] Alternatively, partial sequences of the components may be obtained using standard biochemical purification and microsequencing techniques. For example, purified **complex** as described above may be run on an SDS-PAGE gel and individual bands may be isolated and subjected to protein microsequencing. DNA sequences encoding components may then be prepared by amplification from a suitable human cDNA library, using polymerase chain reaction (PCR) and methods well known to those of ordinary skill in the art. For example, an adapter-ligated cDNA library prepared from a cell line or tissue that expresses **IKK** signalsome (such as HeLa or Jurkat cells) may be screened using a degenerate 5' specific forward primer and an adapter-specific primer. Degenerate oligonucleotides may also be used to screen a cDNA library, using methods well known to those of ordinary skill in the art. In addition, known proteins may be identified via Western blot analysis using specific antibodies.

Detail Description Paragraph - DETX (13):

[0058] Particularly preferred components of **IKK** signalsome are I.kappa.B kinases. An I.kappa.B kinase may be identified based upon its ability to phosphorylate one or more I.kappa.B proteins, which may be readily determined using the representative kinase assays described herein. In general, an I.kappa.B kinase is incorporated into an **IKK** signalsome, as described herein, prior to performing such assays, since an I.kappa.B kinase that is not **complex**-associated may not display the same phosphorylation activity as **complex**-associated I.kappa.B kinase. As noted above, an I.kappa.B kinase within an **IKK** signalsome specifically phosphorylates I.kappa.B.alpha. at residues S32 and S36, and phosphorylates I.kappa.B.beta. at residues 19 and 23, in response to specific stimuli.

Detail Description Paragraph - DETX (22):

[0067] In one aspect of the present invention, an **IKK** signalsome and/or one or more components thereof may be used to identify modulating agents, which may be antibodies (e.g., monoclonal), polynucleotides or other drugs, that inhibit or stimulate signal transduction via the NF-.kappa.B cascade. Modulation includes the suppression or enhancement of NF-.kappa.B activity. Modulation may also include suppression or enhancement of I.kappa.B phosphorylation or the stimulation or inhibition of the ability of activated (i.e., phosphorylated) **IKK** signalsome to phosphorylate a substrate. Compositions that inhibit NF-.kappa.B activity by inhibiting I.kappa.B phosphorylation may include one or

more agents that inhibit or block I.kappa.B.alpha. kinase activity, such as an antibody that neutralizes **IKK** signalsome, a competing peptide that represents the substrate binding domain of I.kappa.B kinase or a phosphorylation motif of I.kappa.B, an antisense polynucleotide or ribozyme that interferes with transcription and/or translation of I.kappa.B kinase, a molecule that inactivates **IKK** signalsome by binding to the **complex**, a molecule that binds to I.kappa.B.alpha. and prevents phosphorylation by **IKK** signalsome or a molecule that prevents transfer of phosphate groups from the kinase to the substrate. Within certain embodiments, a modulating agent inhibits or enhances the expression or activity of **IKK-1** and/or **IKK-2**.

Detail Description Paragraph - DETX (25):

[0070] In another aspect of the present invention, **IKK** signalsome or I.kappa.B kinase may be used for phosphorylating an I.kappa.B such as I.kappa.B.alpha. (or a derivative or variant thereof) so as to render it a target for ubiquitination and subsequent degradation. I.kappa.B may be phosphorylated in vitro by incubating **IKK** signalsome or I.kappa.B kinase with I.kappa.B in a suitable buffer for 30 minutes at 30.degree. C. In general, about 0.01 .mu.g to about 9 .mu.g of I.kappa.B kinase **complex** is sufficient to phosphorylate from about 0.5 .mu.g to about 2 .mu.g of I.kappa.B. Phosphorylated substrate may then be purified by binding to GSH-sepharose and washing. The extent of substrate phosphorylation may generally be monitored by adding [.gamma.-.sup.32P]ATP to a test aliquot, and evaluating the level of substrate phosphorylation as described herein.

Detail Description Paragraph - DETX (34):

[0079] In another aspect, the present invention provides methods for detecting the level of stimulus-inducible I.kappa.B kinase activity in a sample. The level of I.kappa.B kinase activity may generally be determined via an immunokinase assay, in which **IKK** signalsome is first immunoprecipitated with an antibody that binds to the **complex**. The immunoprecipitated material is then subjected to a kinase assay as described herein. Substrate specificity may be further evaluated as described herein to distinguish the activity of a stimulus-inducible I.kappa.B kinase **complex** from other kinase activities.

Detail Description Paragraph - DETX (39):

[0084] Monoclonal antibodies specific for an **IKK** signalsome or a component thereof may be prepared, for example, using the technique of Kohler and Milstein, Eur. J. Immunol. 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (i.e., reactivity with the **complex** and/or component of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine,

aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Detail Description Paragraph - DETX (48):

[0090] This example illustrates the recruitment of NF.kappa.B into a protein **complex (the IKK** signalsome) containing I.kappa.B kinase and other signaling proteins.

Detail Description Paragraph - DETX (50):

[0092] As shown in FIG. 1A, I.kappa.B.alpha. in cell extracts from unstimulated cells eluted with an apparent molecular weight of .about.300 kDa (lanes 5-7), consistent with the chromatographic properties of the inactive NF.kappa.B-I.kappa.B **complex** (Baeuerle and Baltimore, Genes Dev. 3:1689-98, 1989). In contrast, phosphorylated I.kappa.B.alpha. (from cells stimulated for periods too short to permit complete degradation of the protein) migrated at .about.600 kDa on the same chromatography columns (lanes 2, 3). This difference in migration was specific for I.kappa.B, since analysis of the same fractions indicated that the Jun N-terminal kinases JNK1 and JNK2 migrated with low apparent molecular weight and showed no difference in chromatographic behavior between stimulated and unstimulated cells. Stimulation-dependent recruitment of I.kappa.B into this larger protein **complex** corresponded with the appearance of phosphorylated I.kappa.B, suggesting that the **complex** contained the specific I.kappa.B kinases that mediate I.kappa.B phosphorylation. These results demonstrate that that NF.kappa.B activation involves recruitment into a protein **complex (the IKK** signalsome) containing I.kappa.B kinase and other signaling proteins.

Detail Description Paragraph - DETX (58):

[0096] This Example illustrates an alternate preparation of an **IKK** signalsome, and the characterization of the **complex**.

Detail Description Paragraph - DETX (84):

[0120] Of a large panel of antibodies tested, one of three anti-MKP-1 antibodies efficiently co-immunoprecipitated an inducible I.kappa.B kinase activity from HeLa cells as well as primary human umbilical vein endothelial cells (HUVEC). The co-immunoprecipitated kinase (**IKK** signalsome kinase) was inactive in unstimulated HeLa cells, but was rapidly activated within minutes of TNF.alpha. stimulation (FIG. 8A, top panel). The **IKK** signalsome kinase did not phosphorylate a mutant GST-I.kappa.B.alpha. protein in which serine residues 32 and 36 had been mutated to threonine (FIG. 8A top panel, even-numbered lanes). Activation of the signalsome kinase was maximal at 5 minutes and declined thereafter, a time course consistent with the time course of I.kappa.B.alpha. phosphorylation and degradation under the same conditions (FIG. 8A, bottom panel). As expected, the signalsome I.kappa.B kinase was also activated by stimulation of cells with IL-1 or PMA (FIG. 8B, lanes 1-4); moreover, its activity was inhibited in cells treated with TPCK, a known

inhibitor of NF.kappa.B activation (FIG. 8B, lane 7). Additionally, the **IKK** signalsome kinase specifically phosphorylated full-length wild-type I.kappa.B.alpha., but not a mutant I.kappa.B.alpha. bearing the serine 32, 36 to alanine mutations, in the context of a physiological RelA-I.kappa.B.alpha. **complex** (FIG. 8C, lanes 3, 4). Together these results indicate that the anti-MKP-1 antibody co-immunoprecipitated the **IKK** signalsome. The signalsome-associated I.kappa.B kinase met all the criteria expected of the authentic I.kappa.B kinase and had no detectable I.kappa.B.alpha. C-terminal kinase activity.

Detail Description Paragraph - DETX (99):

[0133] This example illustrates the absence of detectable free ubiquitin with a **IKK** signalsome prepared as in Example 3. Standard western blot procedures were performed (Amersham Life Science protocol, Arlington Heights, Ill.). 100 ng ubiquitin, 10 ng ubiquitin and 20 ul purified I.kappa.B.alpha. kinase **complex** was subjected to 16% Tricine SDS-PAGE (Novex, San Diego, Calif.), transferred to Hybond ECL Nitrocellulose membrane (Amersham Life Science, Arlington Heights, Ill.), and probed with antibodies directed against ubiquitin (MAB1510; Chemicon, Temecula, Calif.). The results are shown in FIG. 10. Free ubiquitin could not be detected in the purified I.kappa.B.alpha. kinase preparation (even at very long exposures). The complexes described herein do not require addition of endogenous ubiquitin to detect I.kappa.B.alpha. kinase activity, nor is free ubiquitin a component in the purified I.kappa.B.alpha. kinase preparations of the present invention.

Detail Description Paragraph - DETX (113):

[0145] Both **IKK-1 and IKK-2** kinases were active when expressed in wheat germ extracts, since they were capable of autophosphorylation, but they were inactive with respect to phosphorylation of I.kappa.B substrates. Since both autophosphorylation and substrate phosphorylation were intact in rabbit reticulocyte lysates, there appeared to be a direct correlation between the association of **IKK-1 and IKK-2** into a higher order protein **complex** and the presence of specific I.kappa.B kinase activity in **IKK-1 and IKK-2** immunoprecipitates. This higher order **complex** is most likely the **IKK** signalsome itself. Indeed, immunoprecipitation of rabbit reticulocyte lysates with anti-MKP-1 antibody pulls down a low level of active I.kappa.B kinase activity characteristic of the **IKK** signalsome.

Detail Description Paragraph - DETX (114):

[0146] It is clear that the **IKK** signalsome contains multiple protein components in addition to **IKK-1 and IKK-2** (FIG. 11B). Some of these may be upstream kinases such as MEKK-1 (Chen et al., Cell 84:853-62, 1996) or NIK (Malinin, et al., Nature 385:540-44, 1997); others may be adapter proteins that mediate the **IKK**-1:**IKK**-2 interaction. Indeed MEKK-1 copurifies with **IKK** signalsome activity (FIG. 1C), and two other signalsome proteins have been functionally identified. The protein crossreactive with anti-MKP-1 is an intrinsic component of the **IKK** signalsome kinases, since the I.kappa.B kinase activity coprecipitated with this antibody is stable to washes with 2-4 M urea. Moreover, both **IKK-1** immunoprecipitates and MKP-1 immunoprecipitates containing

the **IKK** signalsome (FIG. 8C) contain an inducible RelA kinase whose kinetics of activation parallel those of the I.kappa.B kinase in the same immunoprecipitates. Another strong candidate for a protein in the signalsome **complex** is the E3 ubiquitin ligase that transfers multiubiquitin chains to phosphorylated I.kappa.B (Hershko et al., Annu. Rev. Biochem. 61:761-807, 1992).

Detail Description Paragraph - DETX (115):

[0147] These results indicate that **IKK-1 and IKK-2** are functional kinases within the **IKK** signalsome, which mediate I.kappa.B phosphorylation and NF.kappa.B activation. Appropriate regulation of **IKK-1 and IKK-2** may require their assembly into a higher order protein **complex,** which may be a heterodimer facilitated by adapter proteins, the complete **IKK** signalsome, or some intermediate subcomplex that contains both **IKK-1 and IKK-2**.

US-PAT-NO: 6642215

DOCUMENT-IDENTIFIER: US 6642215 B2

TITLE: Method of modulating NF-kB activity

DATE-ISSUED: November 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Madsen; Mogens Winkel	Virum	N/A	N/A	DK
Olsen; Lone Stengelsh.o slashed.j	Glostrup	N/A	N/A	DK

APPL-NO: 10/ 153800

DATE FILED: May 24, 2002

PARENT-CASE:

This application claims priority on provisional Application No. 60/292,927 filed on May 24, 2001, the entire contents of which are hereby incorporated by reference.

US-CL-CURRENT: 514/89, 514/344 , 514/346 , 514/352

ABSTRACT:

A method of modulating the level of activated, NF-.kappa.B in cells by contacting cells with a cyanoguanidine compound of general formula I ##STR1##

wherein n is 0, 1 or 2; each R independently represents halogen, trifluoromethyl, hydroxy, C.sub.1-4 alkyl, C.sub.1-4 alkoxy, C.sub.1-4 alkoxycarbonyl, nitro, cyano, amino, sulfo or carboxy groups; Q is a straight or branched, saturated or unsaturated C.sub.4-20 divalent hydrocarbon radical; X is a bond, O, S, amino, carbonyl, carbonylamino, aminocarbonyl, oxycarbonyloxy, oxycarbonyl, carbonyloxy, aminocarbonyloxy, aminothiocarbonyloxy, oxycarbonylamino or oxythiocarbonylamino; A is di-(C.sub.1-4 alkoxy)phosphinoyloxy, C.sub.1-4 alkoxycarbonyl, C.sub.1-4 alkoxycarbonylamino, saturated or unsaturated C.sub.3-12 carbocyclic ring or C.sub.3-12 heterocarbocyclic ring optionally substituted with one or more R.sub.1 ; R.sub.1 being independently selected from the group consisting of halogen, trifluoromethyl, hydroxy, C.sub.1-4 alkyl, C.sub.1-4 alkoxy, C.sub.1-4 alkoxycarbonyl, nitro, cyano, amino, carboxy, sulfo, carboxamido, sulfamoyl or C.sub.1-4 hydroxyalkyl; or a pharmaceutically acceptable salt, N-oxide or N-substituted prodrug thereof, in an amount effective to modulate the activity of IKK.

38 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Brief Summary Text - BSTX (2):
The present invention relates to methods of modulating the activity of NF-.kappa.B and to methods of inhibiting the I.kappa.B **complex (IKK)** using cyanoguanidine derivatives.

Brief Summary Text - BSTX (13):
At the cellular level it is well recognised that nuclear factor .kappa.B (NF-.kappa.B) plays a pivotal role in apoptosis. It is also described that an NF-.kappa.B inhibitor, I.kappa.B, and an I.kappa.B kinase **complex, IKK,** control the level of activated NF.kappa.B [Levkau, 1, 227-233, 1999; Wang, Science, 274, 784-787, 196; Madrid, Molecular and Cellular Biology, 5, 1626-1638, 2000]. Accordingly, the NF-.kappa.B-I.kappa.B-**IKK** system has been suggested as a target in the treatment of neoplastic diseases.

Brief Summary Text - BSTX (23):
In a still further aspect, the invention relates to a method of inhibiting the **IKK complex** by contacting cells with a compound of general formula I, as defined above, in an amount effective to inhibit **IKK**.

Detailed Description Text - DETX (25):
NF-.kappa.B is a member of the Rel family of transcription factors which are ubiquitous in animal cells. Rel proteins can form dimers, the most common of which is designated NF-.kappa.B, NF-.kappa.S is a p50/p65 heterodimer which can activate transcription of genes containing the appropriate .kappa.B binding site. In non-stimulated cells, NF-.kappa.B is maintained in the cytoplasm by interaction with NF-.kappa.B inhibiting proteins, the I.kappa.Bs. In response to cell stimulation by e.g. anti-neoplastic drugs or ionising radiation an I.kappa.B kinase **complex (IKK)** is rapidly activated and phosporylates two serine residues in the NF-.kappa.B binding domain of I.kappa.B. The phophorylated I.kappa.B is then degraded by a 26S proteasome whereas NF-.kappa.B is spared from degradation and translocates into the nucleus [Wang, Science, 274, 784-787, 1996, Cusak, Cancer Research, 60, 2323-2330, 2000; Karin, Immunology, 12, 2000, 85-98]. NF-.kappa.B is thus always present in the cell, but in an inactivated form in non-stimulated cells. After translocation into the nucleus NF-.kappa.B induces inter alia the anti-apoptotic genes c-IAP1, c-IAP2, TRAF1, TRAF2, Bfl-1/A1, Bcl-X.sub.L and Mn-SOD [Patel, Oncogene, 19, 2000, 4159-41699], which bring about resistance in the cells to apoptosis. This effect is referred to as the anti-apoptotic effect of NF-.kappa.B, and the effect may be quantified by measuring the expression of gene products encoded by any of said genes, by any suitable means known in the art, in the presence and absence of compounds modulating the level of activated NK-.kappa.B. Any compound capable of reducing the transcription of one or more

of said genes to a level of less than about 50%, e.g. less than about 30%, such as less than about 20% of the level in the absence of said compound is said to reduce the anti-apoptotic effect of NK-.kappa.B. Anti-neoplastic drugs and ionising radiation thus induce resistance in the cells to the treatments, which render them ineffective. Accordingly, activated NF-.kappa.B is a key factor in induced resistance in e.g. cancer cells to chemotherapeutic drugs and/or to ionising radiation. This is further supported by the fact that constitutively activated NF-.kappa.B is found in cells from resistant cancer tumours [Patel, Oncogene, 19, 4159-4169, 2000]. Regardless of reduced resistance to any treatment, a reduction of the level of activated NF-.kappa.B in the cell, e.g. by controlling the activity of **IKK,** will reduce the expression levels of genes encoding for anti-apoptotic factors, thereby inducing apoptosis in the cells [Schwartz, Surgical Oncology, 8, 1999, 143-153].

Detailed Description Text - DETX (28):

The I.kappa.B kinase **complex (IKK)** consist of three subunits, namely IKK.alpha., IKK.beta. and IKK.gamma., with a combined molecular weight of 900 kDa. IKK.alpha. and IKK.beta. both exhibit I.kappa.B kinase activity and phophorylate I.kappa.B, whereas IKK.gamma. is a regulatory subunit. IKK.alpha. is 85 kDa protein and IKK.beta. is a 87 kDa protein, and the two subunits show a large degree of homology. Whereas both IKK.alpha. and IKK.beta. are catalytically active, it has surprisingly been shown that only IKK.beta. is essential for **IKK** phosphorylation of I.kappa.B. It has been found by the present inventors that compounds of general formula I are effective as inhibitors of IKK.beta. in particular.

Detailed Description Text - DETX (90):

IKK activity: Upon cellular activation by extracellular stimuli, I.kappa.B proteins are phosphorylated by a large I.kappa.B kinase **complex**. An in vitro **IKK** activity assay was established to evaluate a possible effect of compound A on the **IKK** activity. The THP-1 cells were stimulated with 1 .mu.g/ml LPS for 12 min, and then the cells were lysed and immunoprecipitated by an **IKK** antibody. The purified **IKK** was then pretreated with various concentrations of compound A ranging from 10.sup.-11 to 10.sup.-5 M for 30 min. prior to the **IKK** activity assay (FIG. 4). A "chemical zero-point" was introduced by treating the LPS-activated **IKK** with the IKK.beta. inhibitor myricetin (20 .mu.M) (S. H. Tsai et al., supra) to overcome the problem with a prestimulated kinase. A clear dose-response was observed as illustrated by the decrease of GST-I.kappa.B.alpha. phosphorylation in the compound A-treated samples. Four independent experiments were performed and the results are summarised on the plot (FIG. 4). The IC.sub.50 values range from 0.9 nM to 70 nM with a mean IC.sub.50 value of 8 nM.

US-PAT-NO: 6632638

DOCUMENT-IDENTIFIER: US 6632638 B1

TITLE: Enhanced solubility of recombinant proteins using Uracil DNA glycosylase inhibitor

DATE-ISSUED: October 14, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Snavely; Marshall	Moorpark	CA	N/A	N/A
Klionsky; Lana	Ventura	CA	N/A	N/A

APPL-NO: 09/ 715521

DATE FILED: November 17, 2000

US-CL-CURRENT: 435/69.1, 435/69.7 , 435/71.1 , 435/71.2 , 530/350 , 530/351 , 536/23.1 , 536/23.4

ABSTRACT:

Disclosed are methods for improving the solubility of a protein of interest produced recombinantly by expressing the protein of interest as a fusion protein with Uracil DNA glycosylase inhibitor (UGI).

9 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

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Other Reference Publication - OREF (5):

Zandi, et al., "The IkB Kinase **Complex (IKK)** Contains Two Kinase Subunits, IKK.alpha. and IKK.beta., Necessary for IkB Phosphorylation and NF-kB Activation", Cell, vol. 91, pp. 243-252 (1997).

US-PAT-NO: 6630312

DOCUMENT-IDENTIFIER: US 6630312 B2

TITLE: Method for identifying compounds for treatment of insulin resistance

DATE-ISSUED: October 7, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Shoelson; Steven	Natick	MA	N/A	N/A

APPL-NO: 09/ 776432

DATE FILED: February 2, 2001

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part application of and claims priority to U.S. application Ser. No. 09/636,150, now U.S. Pat. No. 6,468,755 filed on Aug. 10, 2000, and U.S. Provisional Application Ser. No. 60/148,037, filed Aug. 10, 1999, the contents of which are incorporated herein by reference.

US-CL-CURRENT: 435/7.1, 435/174 , 435/7.8 , 514/2

ABSTRACT:

The invention features a method of identifying, evaluating or making a compound or agent, e.g., a candidate compound or agent, for treatment of a disorder characterized by insulin resistance. The method includes evaluating the ability of a compound or agent to bind IKK-.beta. or modulate IKK-.beta. activity, to thereby identify a compound or agent for the treatment of a disorder characterized by insulin resistance. The invention also features compounds for treating insulin resistance identified by such methods, and methods of treating a subject having a disorder characterized by insulin resistance by administering such agents.

9 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Brief Summary Text - BSTX (14):

In a preferred embodiment, the ability of a test compound to bind **IKK**-.beta. can be determined by detecting the formation of a **complex** between **IKK**-.beta. and the compound. The presence of the compound in **complex** indicates the ability to bind **IKK**-.beta..

Brief Summary Text - BSTX (25):

In a preferred embodiment, the ability of a test compound to bind **IKK**-.beta. can be determined by detecting the formation of a **complex** between **IKK**-.beta. and the compound. The presence of the compound in **complex** indicates the ability to bind **IKK**-.beta..

Detailed Description Text - DETX (31):

Culture cells were used as follows to investigate the mechanisms relating to salicylate treatment to the in vivo reversal of insulin resistance. TNF-.alpha. treatment of 3T3-L1 adipocytes induced `insulin resistance`, as judged by significant decreases in insulin-stimulated tyrosine phosphorylation of IR .beta.-subunit (42.+-.11%) and IRS-1 (37.+-.9%). TNF-.alpha. mediated `insulin resistance` was reversed by pretreatment with high-dose (5 mM) aspirin. IR and IRS-1 phosphorylation levels were restored to 126.+-.24% and 136.+-.35%, respectively, compared to untreated controls; IR and IRS-1 protein levels were unchanged in TNF-.alpha. and aspirin-treated cells. TNF-.alpha. activates a cascade of adapters and kinases, including TRADD, RIP, TRAF2, and TAB1, which act upstream of JNK, p38 MAPK, and the **IKK complex**. Okadaic acid and calyculin A, two phosphatase inhibitors, also activate IKK.beta. (DiDonato et al. (1997) Nature 388:548; Harhaj & Sun (1997) J Biol Chem 272:5409), but without activating upstream elements in the TNF-.alpha. signaling cascade. Okadaic acid and calyculin A also induce `insulin resistance` in isolated tissues and cultured cells (Robinson et al (1993) Am J Physiol 265:E36; Paz et al (1997) J. Biol Chem 272:29911). Therefore, it was determined whether aspirin would reverse `insulin resistance` caused by these inhibitors. Marked reductions in insulin-stimulated IR (29.+-.12%) and IRS-1 (16.+-.2%) tyrosine-phosphorylation were prevented by incubating the cells with high-dose aspirin (109.+-.15% and 93.+-.25%, respectively). Notably, the reduced electrophoretic mobility of IRS-1 due to calyculin A-induced phosphorylation was reversed with aspirin, further suggesting that aspirin's ability to reverse insulin resistance might occur through reduced Ser/Thr phosphorylation of components in the insulin signaling cascade.

Detailed Description Text - DETX (35):

TNF-.alpha. does not appear to contribute to insulin resistance in type 2 diabetes and syndrome X, as biological blockers of TNF-.alpha. do not alter insulin sensitivity (Ofei et al. (1996) Diabetes 45:881; Paquot et al. (2000) J Clin Endocrinol Metab 85:1316). However, TNF-.alpha. is a potential mediator of acquired insulin resistance (Lang et al. (1992) Endocrinology 130:43; Feinstein et al. (1993) J Biol Chem 268:26055; Hotamisligil et al. (1993) Science 259:87; Hotamisligil et al. (1994) J Clin Invest 94:1543). TNF-.alpha. activates the **IKK complex**. TNF-.alpha. treatment of untransfected 293 cells

reduced insulin-stimulated IR activation to 29.+-.2% of untreated controls. Expression of kinase deficient, dominant inhibitory IKK.alpha.(K44A) or IKK.beta.(K44A) reversed TNF-.alpha.-inhibited IR activation. In fact, dominant-inhibitory IKK.beta. caused a 60% increase in insulin-stimulated IR tyrosine-phosphorylation over controls, whether or not cells had been treated with TNF-.alpha.. Similar effects were seen with AKT. TNF-.alpha. treatment reduced AKT activation (18.+-.15%), and this was reversed by IKK.beta.(K44A) expression (174.+-.38%). Active **IKK** kinases thus mediate 'insulin resistance' in cultured cells, and the inactive kinases act as dominant inhibitors to block TNF-.alpha. induced insulin resistance. The consistent ability of dominant-inhibitory IKK.beta. to elevate IR signaling well above the normal level indicates that **IKK** inhibits insulin signaling even in the absence of TNF-.alpha.. There is in vivo support for this, as well. Fa/+ rats and ob/+ mice (see FIG. 1) and Sprague-Dawley rats that are not insulin resistant, obese, or diabetic, show increased insulin sensitivity in response to aspirin treatment.

Other Reference Publication - OREF (2):

Rothwarf et al., "**IKK**-.gamma. is an essential regulatory subunit of the IkB kinase **complex**", Nature, 395-297-300, Sep., 1998.

US-PAT-NO: 6576444

DOCUMENT-IDENTIFIER: US 6576444 B2

TITLE: IRAK3 polynucleotides

DATE-ISSUED: June 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cao; Zhaodan	South San Francisco	CA	N/A	N/A

APPL-NO: 09/ 863549

DATE FILED: May 22, 2001

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a divisional application of and claims priority under 35 U.S.C. .sctn.120 to U.S. Ser. No. 09/135,232, filed Aug. 17, 1998, now U.S. Pat. No. 6,262,228, which is incorporated herein by reference.

US-CL-CURRENT: 435/69.1, 424/94.1 , 435/194 , 435/252.3 , 435/320.1 , 435/471 , 536/23.5

ABSTRACT:

The invention provides methods and compositions relating to a novel kinase, IRAK3. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IRAK3 encoding nucleic acids or purified from human cells. The invention provides isolated IRAK3 hybridization probes and primers capable of specifically hybridizing with the disclosed IRAK3 genes, IRAK3-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

8 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (5):

Interleukin 1 (IL-1) receptor associated kinase (IRAK) functions as an intracellular signal transducer for the pro-inflammatory cytokine IL-1. IL-1 treatment of cells induces the **complex** formation of the two IL-1 receptor

chains, IL-1R1 and IL-1RAcP, which recruits an adaptor molecule designated as MyD88 which binds to IRAK. IRAK is subsequently phosphorylated, released from the receptor **complex** to interact with TRAF6. TRAF6 triggers either the NIK/**IKK** kinase cascade to activate the transcription factor NF-.kappa.B or an undefined kinase cascade to activate the transcription factor AP-1. Both transcription factors regulate large numbers of genes that regulate immune and inflammatory responses.

US-PAT-NO: 6576439

DOCUMENT-IDENTIFIER: US 6576439 B1

TITLE: IKK3 kinase

DATE-ISSUED: June 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Takemoto; Yoshihiro	Tsukuba	N/A	N/A	JP
Sakai; Yutaka	Tsukuba	N/A	N/A	JP
Hashimoto; Yasuhiro	Shinagawa-Ku	N/A	N/A	JP

APPL-NO: 09/ 868758

DATE FILED: June 21, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9828704	December 24, 1998

PCT-DATA:
APPL-NO: PCT/JP99/07286
DATE-FILED: December 24, 1999
PUB-NO: WO00/39308
PUB-DATE: Jul 6, 2000
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/15, 435/194 , 435/252.3 , 435/320.1 , 435/325 , 435/6

ABSTRACT:

This invention relates to an IKK kinase protein, IKK3, nucleotides coding for it vectors and host cells containing the same and methods for screening for modulators of said IKK3 protein for treatment of conditions involving inflammation.

3 Claims, 25 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

---------- KWIC ---------

Brief Summary Text - BSTX (6):

Several groups found. that two kinases termed **IKK1 and IKK2** (also known as IKK.alpha. and IKK.beta.), were the subunits of the kinase **complex**. The groups showed that the **IKKs** immunoprecipitates, derived from the TNF.alpha. or IL-1 stimulated cells are able to phosphorylate IkB in vitro. In addition to these observations, two groups reported that **IKK1 and IKK2** purified from insect cells are able to phosphorylate IkB in vitro. These results suggested that **IKK** directly phosphorylates IkBs. The over expression of anti-sense **IKK1,** kinase-inactive **IKK1 or IKK2** resulted in the inhibition of NF-kB activation mediated by TNF.alpha. and IL-1. These results suggest that **IKKs** are critical kinases in the NF-kB activation pathway (May and Ghosh, 1998, Immunol. Today 19, 80-88; Stancovski and Baltimore, 1997, Cell, 91, 299-302). It has, however, not been understood how upstream signals are transmitted to the kinase **complex,** or whether different kinase complexes might exist to phosphorylate distinct IkBs.

Brief Summary Text - BSTX (7):

NEMO (NF-kB essential modifier) and IKK.gamma. (human homologue of the mouse NEMO) were isolated from purified **IKK complex,** and the inhibition of NEMO/IKK.gamma. gene expression impaired the cytokine induced NF-kB activation via **IKK1 and IKK2**. In NEMO deficient cells, smaller complexes of Mr 3,000-4,000 are formed, though the normal **complex** is Mr 7,000-9,000, suggesting that NEMO/IKK.gamma. physically link IkB kinase to upstream activators (Scheidereit, Nature, 1998, 395, 225-226).

Brief Summary Text - BSTX (8):

The **IKK-complex**-associated protein (IKAP) was isolated from the **IKK** complexes. IKAP binds to IkB kinases and NIK and the **complex,** containing three kinases, leads to the maximum phosphorylation of IkB as compared to the **complex** containing one or two kinases. Accordingly, IKAP may act as scaffold proteins that link NIK or other molecules to **IKK1 and IKK2** (Scheidereit, Nature, 1998, 395, 225-226). Accumulating evidence suggests that the **IKK complex** consists of several essential molecules, however, the molecular mechanisms that control the signalling **complex** were not well understood. Therefore, further association molecules were needed to complete the picture.

US-PAT-NO: 6576437

DOCUMENT-IDENTIFIER: US 6576437 B2

TITLE: Stimulus-inducible protein kinase complex and methods of use therefor

DATE-ISSUED: June 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mercurio; Frank	San Diego	CA	N/A	N/A
Zhu; Hengyi	San Diego	CA	N/A	N/A
Barbosa; Miguel	San Diego	CA	N/A	N/A
Li; Jian Wu	San Diego	CA	N/A	N/A
Murray; Brion W.	San Diego	CA	N/A	N/A

APPL-NO: 09/ 844908

DATE FILED: April 27, 2001

PARENT-CASE:

CROSS-REFERENCE TO PRIOR APPLICATION

This is a divisional application of Ser. No. 08/910,820 filed Aug. 13, 1997 now U.S. Pat. No. 6,258,579, which is a continuation-in-part of U.S. patent application No. 08/697,393, filed Aug. 26, 1996 now U.S. Pat. No. 5,972,674.

US-CL-CURRENT: 435/15, 435/194 , 435/68.1

ABSTRACT:

Compositions and methods are provided for treating NF-.kappa.B-related conditions. In particular, the invention provides a stimulus-inducible IKK signalsome, and components and variants thereof. An IKK signalsome or component thereof may be used, for example, to identify antibodies and other modulating agents that inhibit or activate signal transduction via the NF-.kappa.B cascade. IKK signalsome, components thereof and/or modulating agents may also be used for the treatment of diseases associated with NF-.kappa.B activation.

10 Claims, 30 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 28

---------- KWIC ---------

Drawing Description Text - DRTX (11):

FIGS. 8A-8C are autoradiograms depicting the results of immunoblot analyses. In FIG. 8A, the upper panel presents a time course for the induction of signalsome activity. Anti MKP-1 immune precipitates from extracts of HeLa S3 cells stimulated with TNF.alpha. (20 ng/ml) for the indicated times, were assayed for **IKK** signalsome activity by standard immune **complex** kinase assays. 4 .mu.g of either GST I.kappa.B.alpha. 1-54 WT (wildtype) or the GST I.kappa.B.alpha. 1-54 S32/36 to T mutant (S>T) were used as the substrates. In the lower panel, HeLa cell extracts prepared as described in the upper panel were examined by western blot analysis for I.kappa.B.alpha. degradation. I.kappa.B.alpha. supershifting phosphorylation can be seen after 3 and 5 minutes of stimulation followed by the disappearance of I.kappa.B.alpha..

Drawing Description Text - DRTX (13):

FIG. 8C illustrates the ability of **IKK** signalsome to specifically phosphorylate serines 32 and 36 of the I.kappa.B.alpha. holoprotein in the context of a RelA:I.kappa.B.alpha. **complex**. Anti-MKP-1 immunoprecipitates from cell extracts of HeLa S3 cells stimulated with TNF.alpha. (20 ng/ml, 7 min) were examined for their ability to phosphorylate baculoviral expressed RelA:I.kappa.B.alpha. **complex** containing either the I.kappa.B.alpha. WT (lane 3) or I.kappa.B.alpha. S32/36 to A mutant (lane 4) holoprotein. The specific substrates used are indicated on the top. Positions of the phosphorylated substrates are indicated by arrows to the left of the panel.

Drawing Description Text - DRTX (16):

FIG. 10 is an autoradiogram showing the results of a western blot analysis of the level of ubiquitin within a stimulus-inducible IkB kinase **complex**. Lane 1 shows the detection of 100 ng ubiquitin, Lane 2 shows 10 ng ubiquitin and Lane 3 shows 3.4 .mu.g of **IKK** signalsome purified through the phenyl superose step (sufficient quantities for 10 kinase reactions). The position of ubiquitin is shown by the arrow on the left.

Detailed Description Text - DETX (2):

As noted above, the present invention is generally directed to compositions and methods for modulating (i.e., stimulating or inhibiting) signal transduction leading to NF-.kappa.B activation. In particular, the present invention is directed to compositions comprising an I.kappa.B kinase (**IKK**) signalsome (also referred to herein as a "stimulus-inducible I.kappa.B kinase **complex**" or "I.kappa.B kinase **complex**") that is capable of stimulus-dependent phosphorylation of I.kappa.B.alpha. and I.kappa.B.beta. on the two N-terminal serine residues critical for the subsequent ubiquitination and degradation in vivo. Such stimulus-dependent phosphorylation may be achieved without the addition of exogenous cofactors. In particular, an **IKK** signalsome specifically phosphorylates I.kappa.B.alpha. (SEQ ID NO:1) at residues S32 and S36 and phosphorylates I.kappa.B.beta. (SEQ ID NO:2) at residues S19 and S23. The present invention also encompasses compositions that contain one or more

components of such an **IKK** signalsome, or variants of such components. Preferred components, referred to herein as "**IKK** signalsome kinases" "I.kappa.B kinases" or **IKKs**) are kinases that, when incorporated into an **IKK** signalsome, are capable of phosphorylating I.kappa.B.alpha. at S32 and S36. Particularly preferred components are **IKK-1** (SEQ ID NO:10) and **IKK-2** (SEQ ID NO:9).

Detailed Description Text - DETX (4):

An **IKK** signalsome has several distinctive properties. Such a **complex** is stable (i.e., its components remain associated following purification as described herein) and has a high-molecular weight (about 500-700 kD, as determined by gel filtration chromatography). As shown in FIGS. 3 (A and B) and 4 (A and B), I.kappa.B kinase activity of an **IKK** signalsome is "stimulus-inducible" in that it is stimulated by TNF.alpha. (i.e., treatment of cells with TNF.alpha. results in increased I.kappa.B kinase activity and I.kappa.B degradation) and/or by one or more other inducers of NF-.kappa.B, such as IL-1, LPS, TPA, UV irradiation, antigens, viral proteins and stress-inducing agents. The kinetics of stimulation by TNF.alpha. correspond to those found in vivo. I.kappa.B kinase activity of an **IKK** signalsome is also specific for S32 and S36 of I.kappa.B.alpha.. As shown in FIGS. 5 (A and B) and 6 (A and B), an **IKK** signalsome is capable of phosphorylating a polypeptide having the N-terminal sequence of I.kappa.B.alpha. (GST-I.kappa.B.alpha.1-54; SEQ ID NO:3), but such phosphorylation cannot be detected in an I.kappa.B.alpha. derivative containing threonine substitutions at positions 32 and 36. In addition, I.kappa.B kinase activity is strongly inhibited by a doubly phosphorylated I.kappa.B.alpha. peptide (i.e., phosphorylated at S32 and S36), but not by an unrelated c-fos phosphopeptide that contains a single phosphothreonine. A further characteristic of an **IKK** signalsome is its ability to phosphorylate a substrate in vitro in a standard kinase buffer, without the addition of exogenous cofactors. Free ubiquitin is not detectable in preparations of **IKK** signalsome (see FIG. 10), even at very long exposures. Accordingly an **IKK** signalsome differs from the ubiquitin-dependent I.kappa.B.alpha. kinase activity described by Chen et al., Cell 84:853-62, 1996.

Detailed Description Text - DETX (5):

An **IKK** signalsome may be immunoprecipitated by antibodies raised against MKP-1 (MAP kinase phosphatase-1; Santa Cruz Biotechnology, Inc., Santa Cruz, Calif. #SC-1102), and its activity detected using an in vitro I.kappa.B.alpha. kinase assay. However, as discussed further below, MKP-1 does not appear to be a component of I.kappa.B kinase **complex**. The substrate specificity of the immunoprecipitated **IKK** signalsome is maintained (i.e., there is strong phosphorylation of wildtype GST-I.kappa.B.alpha. 1-54 (SEQ ID NO:3) and GST-I.kappa.B.beta. 1-44 (SEQ ID NO:4), and substantially no detectable phosphorylation of GST-I.kappa.B.alpha. 1-54 in which serines 32 and 36 are replaced by threonines (GST- I.kappa.B.alpha. S32/36 to T; SEQ ID NO:5) or GST-I.kappa.B.beta. 1-44 in which serines 19 and 23 are replaced by alanines (GST-I.kappa.B.beta. 1-44 S19/23 to A; SEQ ID NO:6)).

Detailed Description Text - DETX (8):

Throughout the fractionation, an in vitro kinase assay may be used to monitor the I.kappa.B kinase activity of the **IKK** signalsome. In such an assay, the ability of a fraction to phosphorylate an appropriate substrate (such as I.kappa.B.alpha. (SEQ ID NO:1) or a derivative or variant thereof) is evaluated by any of a variety of means that will be apparent to those of ordinary skill in the art. For example, a substrate may be combined with a chromatographic fraction in a protein kinase buffer containing .sup.32 P .gamma.-ATP, phosphatase inhibitors and protease inhibitors. The mixture may be incubated for 30 minutes at 30.degree. C. The reaction may then be stopped by the addition of SDS sample buffer and analyzed using SDS-PAGE with subsequent autoradiography. Suitable substrates include full length I.kappa.B.alpha. (SEQ ID NO:1), polypeptides comprising the N-terminal 54 amino acids of I.kappa.B.alpha., full length I.kappa.B.beta. (SEQ ID NO:2) and polypeptides comprising the N-terminal 44 amino acids of I.kappa.B.beta.. Any of these substrates may be used with or without an N-terminal tag. One suitable substrate is a protein containing residues 1-54 of I.kappa.B.alpha. and an N-terminal GST tag (referred to herein as GST-I.kappa.B.alpha. 1-54; SEQ ID NO:3). To evaluate the specificity of an I.kappa.B kinase **complex,** I.kappa.B.alpha. mutants containing threonine or alanine residues at positions 32 and 36, and/or other modifications, may be employed.

Detailed Description Text - DETX (9):

Alternatively, an **IKK** signalsome may be prepared from its components which are also encompassed by the present invention. Such components may be produced using well known recombinant techniques, as described in greater detail below. Components of an **IKK** signalsome may be native, or may be variants of a native component (ie., a component sequence may differ from the native sequence in one or more substitutions and/or modifications, provided that the ability of a **complex** comprising the component variant to specifically phosphorylate I.kappa.B.alpha. is not substantially diminished). Substitutions and/or modifications may generally be made in non-critical and/or critical regions of the native protein. Variants may generally comprise residues of L-amino acids, D-amino acids, or any combination thereof. Amino acids may be naturally-occurring or may be non-natural, provided that at least one amino group and at least one carboxyl group are present in the molecule; .alpha.- and .beta.-amino acids are generally preferred. A variant may also contain one or more rare amino acids (such as 4-hydroxyproline or hydroxylysine), organic acids or amides and/or derivatives of common amino acids, such as amino acids having the C-terminal carboxylate esterified (e.g., benzyl, methyl or ethyl ester) or amidated and/or having modifications of the N-terminal amino group (e.g., acetylation or alkoxycarbonylation), with or without any of a wide variety of side-chain modifications and/or substitutions (e.g., methylation, benzylation, t-butylation, tosylation, alkoxycarbonylation, and the like). Component variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids that have minimal influence on the activity of the polypeptide. In particular, variants may contain additional amino acid sequences at the amino and/or carboxy termini. Such sequences may be used, for example, to facilitate purification or detection of the component polypeptide. In general, the effect of one or more substitutions and/or modifications may be evaluated using the representative assays provided herein.

Detailed Description Text - DETX (11):

Alternatively, partial sequences of the components may be obtained using standard biochemical purification and microsequencing techniques. For example, purified **complex** as described above may be run on an SDS-PAGE gel and individual bands may be isolated and subjected to protein microsequencing. DNA sequences encoding components may then be prepared by amplification from a suitable human cDNA library, using polymerase chain reaction (PCR) and methods well known to those of ordinary skill in the art. For example, an adapter-ligated cDNA library prepared from a cell line or tissue that expresses **IKK** signalsome (such as HeLa or Jurkat cells) may be screened using a degenerate 5' specific forward primer and an adapter-specific primer. Degenerate oligonucleotides may also be used to screen a cDNA library, using methods well known to those of ordinary skill in the art. In addition, known proteins may be identified via Western blot analysis using specific antibodies.

Detailed Description Text - DETX (13):

Particularly preferred components of **IKK** signalsome are I.kappa.B kinases. An I.kappa.B kinase may be identified based upon its ability to phosphorylate one or more I.kappa.B proteins, which may be readily determined using the representative kinase assays described herein. In general, an I.kappa.B kinase is incorporated into an **IKK** signalsome, as described herein, prior to performing such assays, since an I.kappa.B kinase that is not **complex**-associated may not display the same phosphorylation activity as **complex**-associated I.kappa.B kinase. As noted above, an I.kappa.B kinase within an **IKK** signalsome specifically phosphorylates I.kappa.B.alpha. at residues S32 and S36, and phosphorylates I.kappa.B.beta. at residues 19 and 23, in response to specific stimuli.

Detailed Description Text - DETX (22):

In one aspect of the present invention, an **IKK** signalsome and/or one or more components thereof may be used to identify modulating agents, which may be antibodies (e.g., monoclonal), polynucleotides or other drugs, that inhibit or stimulate signal transduction via the NF-.kappa.B cascade. Modulation includes the suppression or enhancement of NF-.kappa.B activity. Modulation may also include suppression or enhancement of I.kappa.B phosphorylation or the stimulation or inhibition of the ability of activated (i.e., phosphorylated) **IKK** signalsome to phosphorylate a substrate. Compositions that inhibit NF-.kappa.B activity by inhibiting I.kappa.B phosphorylation may include one or more agents that inhibit or block I.kappa.B.alpha. kinase activity, such as an antibody that neutralizes **IKK** signalsome, a competing peptide that represents the substrate binding domain of I.kappa.B kinase or a phosphorylation motif of I.kappa.B, an antisense polynucleotide or ribozyme that interferes with transcription and/or translation of I.kappa.B kinase, a molecule that inactivates **IKK** signalsome by binding to the **complex**, a molecule that binds to I.kappa.B and prevents phosphorylation by **IKK** signalsome or a molecule that prevents transfer of phosphate groups from the kinase to the substrate. Within certain embodiments, a modulating agent inhibits or enhances the expression or activity of **IKK-1** and/or **IKK-2**.

Detailed Description Text - DETX (25):

In another aspect of the present invention, **IKK** signalsome or I.kappa.B kinase may be used for phosphorylating an I.kappa.B such as I.kappa.B.alpha. (or a derivative or variant thereof) so as to render it a target for ubiquitination and subsequent degradation. I.kappa.B may be phosphorylated in vitro by incubating **IKK** signalsome or I.kappa.B kinase with I.kappa.B in a suitable buffer for 30 minutes at 30.degree. C. In general, about 0.01 .mu.g to about 9 .mu.g of I.kappa.B kinase **complex** is sufficient to phosphorylate from about 0.5 .mu.g to about 2 .mu.g of I.kappa.B. Phosphorylated substrate may then be purified by binding to GSH-sepharose and washing. The extent of substrate phosphorylation may generally be monitored by adding [.gamma.-.sup.32 P]ATP to a test aliquot, and evaluating the level of substrate phosphorylation as described herein.

Detailed Description Text - DETX (34):

In another aspect, the present invention provides methods for detecting the level of stimulus-inducible I.kappa.B kinase activity in a sample. The level of I.kappa.B kinase activity may generally be determined via an immunokinase assay, in which **IKK** signalsome is first immunoprecipitated with an antibody that binds to the **complex**. The immunoprecipitated material is then subjected to a kinase assay as described herein. Substrate specificity may be further evaluated as described herein to distinguish the activity of a stimulus-inducible I.kappa.B kinase **complex** from other kinase activities.

Detailed Description Text - DETX (39):

Monoclonal antibodies specific for an **IKK** signalsome or a component thereof may be prepared, for example, using the technique of Kohler and Milstein, Eur. J. Immunol. 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (i.e., reactivity with the **complex** and/or component of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Detailed Description Text - DETX (48):

This example illustrates the recruitment of NF.kappa.B into a protein **complex (the IKK** signalsome) containing I.kappa.B kinase and other signaling proteins.

Detailed Description Text - DETX (50):

As shown in FIG. 1A, I.kappa.B.alpha. in cell extracts from unstimulated cells eluted with an apparent molecular weight of .about.300 kDa (lanes 5-7), consistent with the chromatographic properties of the inactive NF.kappa.B-I.kappa.B **complex** (Baeuerle and Baltimore, Genes Dev. 3:1689-98, 1989). In contrast, phosphorylated I.kappa.B.alpha. (from cells stimulated for periods too short to permit complete degradation of the protein) migrated at .about.600 kDa on the same chromatography columns (lanes 2, 3). This difference in migration was specific for I.kappa.B, since analysis of the same fractions indicated that the Jun N-terminal kinases JNK1 and JNK2 migrated with low apparent molecular weight and showed no difference in chromatographic behavior between stimulated and unstimulated cells. Stimulation-dependent recruitment of I.kappa.B into this larger protein **complex** corresponded with the appearance of phosphorylated I.kappa.B, suggesting that the **complex** contained the specific I.kappa.B kinases that mediate I.kappa.B phosphorylation. These results demonstrate that that NF.kappa.B activation involves recruitment into a protein **complex (the IKK** signalsome) containing I.kappa.B kinase and other signaling proteins.

Detailed Description Text - DETX (58):

This Example illustrates an alternate preparation of an **IKK** signalsome, and the characterization of the **complex**.

Detailed Description Text - DETX (78):

Of a large panel of antibodies tested, one of three anti-MKP-1 antibodies efficiently co-immunoprecipitated an inducible I.kappa.B kinase activity from HeLa cells as well as primary human umbilical vein endothelial cells (HUVEC). The co-immunoprecipitated kinase (**IKK** signalsome kinase) was inactive in unstimulated HeLa cells, but was rapidly activated within minutes of TNF.alpha. stimulation (FIG. 8A, top panel). The **IKK** signalsome kinase did not phosphorylate a mutant GST-I.kappa.B.alpha. protein in which serine residues 32 and 36 had been mutated to threonine (FIG. 8A top panel, even-numbered lanes). Activation of the signalsome kinase was maximal at 5 minutes and declined thereafter, a time course consistent with the time course of I.kappa.B.alpha. phosphorylation and degradation under the same conditions (FIG. 8A, bottom panel). As expected, the signalsome I.kappa.B kinase was also activated by stimulation of cells with IL-1 or PMA (FIG. 8B, lanes 1-4); moreover, its activity was inhibited in cells treated with TPCK, a known inhibitor of NF.kappa.B activation (FIG. 8B, lane 7). Additionally, the **IKK** signalsome kinase specifically phosphorylated full-length wild-type I.kappa.B.alpha., but not a mutant I.kappa.B.alpha. bearing the serine 32, 36 to alanine mutations, in the context of a physiological RelA-I.kappa.B.alpha. **complex** (FIG. 8C, lanes 3, 4). Together these results indicate that the anti-MKP-1 antibody co-immunoprecipitated the **IKK** signalsome. The signalsome-associated I.kappa.B kinase met all the criteria expected of the authentic I.kappa.B kinase and had no detectable I.kappa.B.alpha. C-terminal kinase activity.

Detailed Description Text - DETX (87):

This example illustrates the absence of detectable free ubiquitin with a **IKK** signalsome prepared as in Example 3. Standard western blot procedures were performed (Amersham Life Science protocol, Arlington Heights, Ill.). 100 ng ubiquitin, 10 ng ubiquitin and 20 .mu.l purified IkB.alpha. kinase **complex** was subjected to 16% Tricine SDS-PAGE (Novex, San Diego, Calif.), transferred to Hybond ECL Nitrocellulose membrane (Amersham Life Science, Arlington Heights, Ill.), and probed with antibodies directed against ubiquitin (MAB1510; Chemicon, Temecula, Calif.). The results are shown in FIG. 10. Free ubiquitin could not be detected in the purified IkB.alpha. kinase preparation (even at very long exposures). The complexes described herein do not require addition of endogenous ubiquitin to detect IkB.alpha. kinase activity, nor is free ubiquitin a component in the purified IkB.alpha. kinase preparations of the present invention.

Detailed Description Text - DETX (101):

Both **IKK-1 and IKK-2** kinases were active when expressed in wheat germ extracts, since they were capable of autophosphorylation, but they were inactive with respect to phosphorylation of I.kappa.B substrates. Since both autophosphorylation and substrate phosphorylation were intact in rabbit reticulocyte lysates, there appeared to be a direct correlation between the association of **IKK-1 and IKK-2** into a higher order protein **complex** and the presence of specific I.kappa.B kinase activity in **IKK-1 and IKK-2** immunoprecipitates. This higher order **complex** is most likely the **IKK** signalsome itself. Indeed, immunoprecipitation of rabbit reticulocyte lysates with anti-MKP-1 antibody pulls down a low level of active I.kappa.B kinase activity characteristic of the **IKK** signalsome.

Detailed Description Text - DETX (102):

It is clear that the **IKK** signalsome contains multiple protein components in addition to **IKK-1 and IKK-2** (FIG. 11B). Some of these may be upstream kinases such as MEKK-1 (Chen et al., Cell 84:853-62, 1996) or NIK (Malinin, et al., Nature 385:540-44, 1997); others may be adapter proteins that mediate the **IKK**-1:**IKK**-2 interaction. Indeed MEKK-1 copurifies with **IKK** signalsome activity (FIG. 1C), and two other signalsome proteins have been functionally identified. The protein crossreactive with anti-MKP-1 is an intrinsic component of the **IKK** signalsome kinases, since the I.kappa.B kinase activity coprecipitated with this antibody is stable to washes with 2-4 M urea Moreover, both **IKK-1** immunoprecipitates and MKP-1 immunoprecipitates containing the **IKK** signalsome (FIG. 8C) contain an inducible RelA kinase whose kinetics of activation parallel those of the I.kappa.B kinase in the same immunoprecipitates. Another strong candidate for a protein in the signalsome **complex** is the E3 ubiquitin ligase that transfers multiubiquitin chains to phosphorylated I.kappa.B (Hershko et al., Annu. Rev. Biochem. 61:761-807,1992).

Detailed Description Text - DETX (103):

These results indicate that **IKK-1 and IKK-2** are functional kinases within the **IKK** signalsome, which mediate I.kappa.B phosphorylation and NF.kappa.B activation. Appropriate regulation of **IKK-1 and IKK-2** may require their

assembly into a higher order protein **complex,** which may be a heterodimer facilitated by adapter proteins, the complete **IKK** signalsome, or some intermediate subcomplex that contains both **IKK-1 and IKK-2**.

US-PAT-NO: 6562811

DOCUMENT-IDENTIFIER: US 6562811 B1

TITLE: Pyridine derivatives

DATE-ISSUED: May 13, 2003

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APPL-NO: 09/ 956618

DATE FILED: September 18, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	2000-289173	September 22, 2000

US-CL-CURRENT: 514/222.5, 514/226.5 , 514/230.5 , 514/234.2 , 514/248 , 514/300 , 514/303 , 544/10 , 544/117 , 544/127 , 544/2 , 544/279 , 544/362 , 544/48 , 544/91 , 546/119 , 546/122 , 546/123

ABSTRACT:

Pyridine compounds of general formula: ##STR1##

wherein --R.sup.1 represents ##STR2##

in which R.sup.11 is hydrogen, C.sub.1-6 alkyl, halogen, hydroxy, C.sub.1-12 alkoxy, nitro, amino, C.sub.1-6 alkylsulfonylamino, C.sub.1-6 alkoxycarbonyl, C.sub.1-6 alkylamino, di(C.sub.1-6 alkyl)amino, C.sub.1-6 alkanoylamino, phenyl C.sub.1-6 alkylamino, phenylsulfonylamino, or --O--(CH.sub.2).sub.n --R.sup.111 ; R.sup.2 represents hydrogen or halogen; R.sup.3 represents hydrogen, --CR.sup.31 R.sup.32 R.sup.33, or --NR.sup.34 R.sup.35 ; R.sup.4 is hydrogen,

carbamoyl, CN, carboxyl, etc.; R.sup.5 is amino, C.sub.1-6 alkylamino, di C.sub.1-6 alkylamino, etc. or salt thereof. The compound has an excellent anti-inflammatory activity, and other biological activity.

12 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (10):

Extensive research during the past years led to the identification of an I.kappa.B kinase (**IKK) complex** as being responsible for the signal-induced I.kappa.B phosphorylation ((Mercurio, F., and Manning, A. M. (1999) Current Opinion in Cell Biology, 11:226-232), (Mercurio, F., and Manning, A. M. (1999) Oncogene, 18:6163-6171), (Barnkett, M., and Gilmore T. D. (1999) Oncogene 18, 6910-6924), (Zandi, E., and Karin, M., (1999) 19:4547-4551), (Israel, A., (2000) trends in CELL BIOLOGY 10:129-133), and (Hatada, E. N, et al. (2000) Current Opinion in Immunology, 12:52-58)). This **complex** is most likely the site of integration of all of the different stimuli leading to NF-.kappa.B activation. The **IKK-complex** (molecular weight 700-900 kDa) is composed of various proteins including two homologous I.kappa.B kinases, called **IKK**-.alpha. and **IKK**-.beta., an upstream kinase, NIK which induces NF-.kappa.B, a scaffold protein called IKAP, which tethers together the three kinases, and a regulatory subunit **IKK**-.gamma., which preferentially interacts with **IKK**-.beta..

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	436	ikk$2	USPAT; US-PGPUB	2003/11/04 09:50
2	L2	584058	complex	USPAT; US-PGPUB	2003/11/04 09:50
3	L3	120	1 same 2	USPAT; US-PGPUB	2003/11/04 09:51
4	L4	25	spa-1	USPAT; US-PGPUB	2003/11/04 14:41
5	L5	3	1 and 4	USPAT; US-PGPUB	2003/11/04 14:41

US-PAT-NO: 6607879

DOCUMENT-IDENTIFIER: US 6607879 B1

TITLE: Compositions for the detection of blood cell and immunological response gene expression

DATE-ISSUED: August 19, 2003

INVENTOR-INFORMATION:

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Seilhamer; Jeffrey J.	Los Altos Hills	CA	N/A	N/A

APPL-NO: 09/ 023655

DATE FILED: February 9, 1998

US-CL-CURRENT: 435/6, 435/69.1 , 536/23.1 , 536/24.1 , 536/24.3 , 536/24.31 , 536/24.32 , 536/24.33

ABSTRACT:

The present invention relates to a composition comprising a plurality of polynucleotide probes. The composition can be used as hybridizable array elements in a microarray. The present invention also relates to a method for selecting polynucleotide probes for the composition.

7 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Detailed Description Paragraph Table - DETL (3):
cds. SEQ ID NO: 187 060269 INCYTE INCYTE SEQ ID NO: 188 060309 g1663703 Human mRNA for KIAA0242 gene, partial cds. SEQ ID NO: 189 060779 INCYTE INCYTE SEQ ID NO: 190 060951 INCYTE INCYTE SEQ ID NO: 191 064994 INCYTE INCYTE SEQ ID NO: 192 071177 INCYTE INCYTE SEQ ID NO: 193 073159 INCYTE INCYTE SEQ ID NO: 194 073293 INCYTE INCYTE SEQ ID NO: 195 073345 INCYTE INCYTE SEQ ID NO: 196 073347 INCYTE INCYTE SEQ ID NO: 197 073582 g557875 Mus musculus SKD1 protein mRNA, complete cds. SEQ ID NO: 198 073735 g529642 Human mRNA for leukotriene B4 omega- hydroxylase, complete cds. SEQ ID NO: 199 074398 INCYTE INCYTE SEQ ID NO: 200 074431 INCYTE INCYTE SEQ ID NO: 201 074449 g1791256 Human copine I mRNA, complete cds. SEQ ID NO: 203 075091

g38050 M.fascicularis gene for apolipoprotein A-IV. SEQ ID NO: 204 075169 INCYTE INCYTE SEQ ID NO: 205 078090 INCYTE INCYTE SEQ ID NO: 206 078610 INCYTE INCYTE SEQ ID NO: 207 078771 INCYTE INCYTE SEQ ID NO: 208 078822 INCYTE INCYTE SEQ ID NO: 209 079378 INCYTE INCYTE SEQ ID NO: 210 079479 INCYTE INCYTE SEQ ID NO: 211 079895 INCYTE INCYTE SEQ ID NO: 212 080226 INCYTE INCYTE SEQ ID NO: 213 080231 INCYTE INCYTE SEQ ID NO: 214 080369 INCYTE INCYTE SEQ ID NO: 215 080752 g1913891 Human clone 23652 mRNA sequence. SEQ ID NO: 216 081040 INCYTE INCYTE SEQ ID NO: 217 081583 INCYTE INCYTE SEQ ID NO: 218 084009 g339877 Homo sapiens tripeptidyl peptidase II mRNA, 3' end. SEQ ID NO: 219 084374 INCYTE INCYTE SEQ ID NO: 220 086674 INCYTE INCYTE SEQ ID NO: 221 087031 INCYTE INCYTE SEQ ID NO: 222 087235 g1654324 Human chromosome 5 Mad homolog Smad5 mRNA, complete cds. SEQ ID NO: 223 089993 g184349 Human hypoxanthine phosphoribosyltransferase (HPRT) mRNA SEQ ID NO: 224 098835 g2330739 SPAC1B3.05 hypothetical protein SEQ ID NO: 225 1000787 INCYTE INCYTE SEQ ID NO: 226 1004415 g1707479 H.sapiens mRNA for CRM1 protein. SEQ ID NO: 227 101411 INCYTE INCYTE SEQ ID NO: 228 103585 INCYTE INCYTE SEQ ID NO: 229 103656 INCYTE INCYTE SEQ ID NO: 230 103704 INCYTE INCYTE SEQ ID NO: 231 103933 g2358042 Homo sapiens T-cell receptor alpha delta locus from bases 501613 to 103933 SEQ ID NO: 232 104098 INCYTE INCYTE SEQ ID NO: 233 104159 INCYTE INCYTE SEQ ID NO: 234 104211 INCYTE INCYTE SEQ ID NO: 235 104368 g1022903 Human phosducin-like protein (PhLP) gene, partial cds. intron 2(partial)/exon 3/intron 3 (partial). SEQ ID NO: 236 104451 g2351798 Human clone HM18 monocyte inhibitory receptor precursor mRNA, SEQ ID NO: 237 104731 INCYTE INCYTE SEQ ID NO: 238 104903 INCYTE INCYTE SEQ ID NO: 239 104965 INCYTE INCYTE SEQ ID NO: 240 105088 g2088550 Human hereditary haemochromatosis region, histone 2A-like protein SEQ ID NO: 241 105363 INCYTE INCYTE SEQ ID NO: 242 108082 g1399461 Human serine/threonine-protein kinase PRP4h (PRP4h) mRNA, complete SEQ ID NO: 243 108465 g38014 Human mRNA for zinc finger protein (clone 431) SEQ ID NO: 244 108608 g2078532 Human DNA binding protein FKHL15 (FKHL15) mRNA, complete cds. SEQ ID NO: 245 108762 INCYTE INCYTE SEQ ID NO: 246 108819 INCYTE INCYTE SEQ ID NO: 247 109390 INCYTE INCYTE SEQ ID NO: 248 109451 INCYTE INCYTE SEQ ID NO: 249 109706 INCYTE INCYTE SEQ ID NO: 250 109719 INCYTE INCYTE SEQ ID NO: 251 114110 INCYTE INCYTE SEQ ID NO: 252 114495 g169880 nodulin SEQ ID NO: 253 1216210 g391765 Mouse mRNA for peptidylarginine deiminase, complete cds. SEQ ID NO: 254 1217861 g2443362 Homo sapiens mRNA for STAT induced STAT inhibitor-3, complete cds. SEQ ID NO: 255 1218519 g1864004 Human mRNA for transmembrane protein, complete cds. SEQ ID NO: 256 122762 g2194202 Homo sapiens pescadillo mRNA, complete cds. SEQ ID NO: 257 1234356 g407307 Human 54 kDa protein mRNA, complete cds. SEQ ID NO: 258 1241495 g1944415 Human mRNA for KIAA0235 gene, partial cds. SEQ ID NO: 259 1242547 g1000861 Homo sapiens creatine kinase B mRNA, complete cds. SEQ ID NO: 260 1243040 g1731808 Human mRNA for c-myc binding protein, complete cds. SEQ ID NO: 261 1256257 g793182 Homo sapiens cDNA clone 38356 5' similar to SP:S34291 S34291 CYTOCHROME P-450 - FRUIT FLY SEQ ID NO: 262 1257695 g1817732 Human KIT protein and alternatively spliced KIT protein (KIT) gene, SEQ ID NO: 263 1257906 g2286223 **IKK-a; IKK-a** kinase SEQ ID NO: 264 1260257 g1139592 Mus musculus leptin receptor (Ob-r) mRNA, complete CDs. SEQ ID NO: 265 1261161 * INCYTE INCYTE SEQ ID NO: 266 1265680 g286230 Rat NAP-22 mRNA for acidic membrane protein of rat brain, complete SEQ ID NO: 267 126758 INCYTE INCYTE SEQ ID NO: 268 1268703 g407955 Human membrane-associated protein (HEM-1) mRNA, complete cds. SEQ ID NO: 269 1271822 g189389 Homo sapiens osteogenic protein-2 (OP-2) mRNA, complete CDs. SEQ ID NO: 270 1274145 g263309 Vgr-2 = transforming growth factor- beta

homolog SEQ ID NO: 271 1286844 g1226242 EF-hand Ca2 + binding protein p22 SEQ ID NO: 272 1291208 g642116 Human XRCC1 DNA repair gene, genomic. SEQ ID NO: 273 1292521 g1490514 Rat maspin mRNA, complete cds. SEQ ID NO: 274 1298861 g1229044 C11H1 C.elegans SEQ ID NO: 275 1299537 g1008046 Cytochrome P450 SEQ ID NO: 276 1302907 g401771 Homo sapiens ribosomal protein S6 kinase 2 (RPS6KA2) mRNA, partial cds. SEQ ID NO: 277 1303190 INCYTE INCYTE SEQ ID NO: 278 1305494 g1654001 H.sapiens mRNA for Sop2p-like protein SEQ ID NO: 279 1307464 g473361 vitellogenic carboxypeptidase SEQ ID NO: 280 1307568 g1572817 K08F11 C.elegans SEQ ID NO: 281 1310265 g452059 Human insulin-like growth factor binding protein 5 (IGFBP5) mRNA. SEQ ID NO: 282 1317697 g1216525 Human p38-2G4 mRNA, partial cds. SEQ ID NO: 283 131925 INCYTE INCYTE SEQ ID NO: 284 132313 INCYTE INCYTE SEQ ID NO: 285 132537 g1638827 Human DNA sequence from BAC 397C4 on chromosome 22q12-qter contains ESTs and STS. SEQ ID NO: 286 1326793 g220391 Mouse gene for cytokeratin endo A. SEQ ID NO: 287 133060 BL01066B Hypothetical YBR002c family proteins. SEQ ID NO: 288 133089 g2315986 Human high-affinity copper uptake protein (hCTR1) mRNA, complete cds SEQ ID NO: 289 133107 INCYTE INCYTE SEQ ID NO: 290 1339742 g1575664 Rat calcium-activated potassium channel SEQ ID NO: 291 1340453 g473406 Mus musculus Hsp70-related NST-1 (hsr.1) mRNA, complete cds SEQ ID NO: 292 1341948 g1209752 Cybb; gp91phox SEQ ID NO: 293 1344641 g598955 Human mRNA for hepatoma-derived growth factor, complete cds. SEQ ID NO: 294 134481 g1857330 Human SPS1/STE20 homolog KHS1 mRNA, complete cds. SEQ ID NO: 295 1346478 g1667346 T13F2 C.elegans SEQ ID NO: 296 1347577 g1389723 Mouse transcription factor MMUSF (USF) gene, exons 1-10 complete cds SEQ ID NO: 297 1347596 g575457 CDC40; Cdc40p SEQ ID NO: 298 134898 INCYTE INCYTE SEQ ID NO: 299 134902 g484295 Rat mRNA for Synaptotagmin III, complete cds SEQ ID NO: 300 1350210 g1127832 Human heat shock protein hsp40 homolog mRNA, complete cds. SEQ ID NO: 301 1353065 g182736 Human cerebellar degeneration- associated protein mRNA, complete cds SEQ ID NO: 302 135360 INCYTE INCYTE SEQ ID NO: 303 135394 g1137697 Homo sapiens cDNA clone 261714 5' similar to SP:BYR2_SCHPO P28829 PROTEIN KINASE BYR2 SEQ ID NO: 304 135651 g180617 Homo sapiens collagenase mRNA, complete cds. SEQ ID NO: 305 1362601 g886049 Human Ich-2 cysteine protease mRNA, complete cds. SEQ ID NO: 306 1363543 g183007 Human glucocerebrosidase mRNA, complete cds. SEQ ID NO: 307 136466 g1518917 Human DNAJ homolog (DNAJW) gene, complete cds. SEQ ID NO: 308 1378524 g2305263 Homo sapiens chemokine receptor X (CKRX) mRNA, complete cds. SEQ ID NO: 309 1382605 g1794218 Human 150 kDa oxygen-regulated protein ORP150 mRNA, complete cds. SEQ ID NO: 310 139332 INCYTE INCYTE SEQ ID NO: 311 139645 INCYTE INCYTE SEQ ID NO: 312 140055 g1914848 Mus musculus WW domain binding protein 3 mRNA, partial cds. SEQ ID NO: 313 140290 INCYTE INCYTE SEQ ID NO: 314 140314 INCYTE INCYTE SEQ ID NO: 315 140340 INCYTE INCYTE SEQ ID NO: 316 1404269 g1517896 Human renal cell carcinoma antigen RAGE-1 mRNA, complete putative SEQ ID NO: 317 1405467 g220391 Mouse ferritin heavy chain gene, complet SEQ ID NO: 318 1406078 g340038 Human protein tyrosine kinase related mRNA sequence. SEQ ID NO: 319 140628 INCYTE INCYTE SEQ ID NO: 320 140652 INCYTE INCYTE SEQ ID NO: 321 140693 INCYTE INCYTE SEQ ID NO: 322 140704 INCYTE INCYTE SEQ ID NO: 323 140809 INCYTE INCYTE SEQ ID NO: 324 141286 INCYTE INCYTE SEQ ID NO: 325 141304 INCYTE INCYTE SEQ ID NO: 326 141389 INCYTE INCYTE SEQ ID NO: 327 141399 g2072422 Human huntingtin interacting protein (HIP1) mRNA, complete cds. SEQ ID NO: 328 1414094 g640037 A20 protein; murine A20 SEQ ID NO: 329 141454 g1019164 Human beta adaptin gene, exons 1-4, and partial cds. SEQ ID NO: 330 141618 INCYTE INCYTE SEQ ID NO: 331 1418681 g825544 unknown. SEQ ID NO: 332 1418802 g396492 H.sapiens mRNA for rod cGMP phosphodiesterase. SEQ ID

NO: 333 1418874 g391694 Hamster mRNA for cyclinB2, complete cds SEQ ID NO: 334 1419118 g2062674 inhibitor of apoptosis protein 1 SEQ ID NO: 335 142456 g1236166 Human DNA sequence from cosmid J30E17, between markers DXS366 and DXS87 on chromosome X contains repeat polymorphism and ribosomal protein L7A. SEQ ID NO: 336 1425434 g1469187 KIAA0132 SEQ ID NO: 337 1427866 g1665772 Human mRNA for KIAA0253 gene, partial cds. SEQ ID NO: 338 1429970 g1718196 Human translation initiation factor eIF-3 p110 subunit gene, complete cds. SEQ ID NO: 339 143092 g1848232 Human DNA-binding protein CPBP (CPBP) mRNA, partial cds. SEQ ID NO: 340 143157 g2460199 Homo sapiens eukaryotic translation initiation factor 3 subunit (p42) SEQ ID NO: 341 1432736 g1632761 Human mRNA for TPRDI, complete cds. SEQ ID NO: 342 143379 g183369 Human glia maturation factor beta mRNA, complete cds. SEQ ID NO: 343 143403 INCYTE INCYTE SEQ ID NO: 344 1439061 g1666070 H.sapiens mRNA for GAR22 protein. SEQ ID NO: 345 1440128 g401766 Homo sapiens growth-arrest-specific protein (gas) mRNA, complete cds. SEQ ID NO: 346 144388 INCYTE INCYTE SEQ ID NO: 347 1444245 g1857460 Human immunoglobulin-like transcript-3 mRNA, complete cds. SEQ ID NO: 348 144484 INCYTE INCYTE SEQ ID NO: 349 144491 INCYTE INCYTE SEQ ID NO: 350 1445507 g2406579 Homo sapiens nuclear VCP-like protein NVLp.1 (NVL.1) mRNA, complete

Detailed Description Paragraph Table - DETL (5):

SEQ ID NO: 492 198126 INCYTE INCYTE SEQ ID NO: 493 199094 INCYTE INCYTE SEQ ID NO: 494 199150 INCYTE INCYTE SEQ ID NO: 495 199173 INCYTE INCYTE SEQ ID NO: 496 199305 INCYTE INCYTE SEQ ID NO: 497 199690 g1184317 Human inhibitor of apoptosis protein 2 mRNA, complete cds. SEQ ID NO: 498 199812 INCYTE INCYTE SEQ ID NO: 499 1999147 g1041680 Rattus norvegicus phospholipase A-2-activating protein (plap) mRNA, complete cds. SEQ ID NO: 500 200015 INCYTE INCYTE SEQ ID NO: 501 200044 g1136395 Human mRNA for KIAA0168 gene, complete cds. gb100pri SEQ ID NO: 502 200097 INCYTE INCYTE SEQ ID NO: 503 200212 INCYTE INCYTE SEQ ID NO: 504 2006402 g1216374 Rat Tclone4 mRNA. SEQ ID NO: 505 200844 INCYTE INCYTE SEQ ID NO: 506 201349 INCYTE INCYTE SEQ ID NO: 507 201358 INCYTE INCYTE SEQ ID NO: 508 201392 BL00257 Bombesin-like peptides family proteins. SEQ ID NO: 509 201507 INCYTE INCYTE SEQ ID NO: 510 2016903 g247306 cytochrome P450 reductase [human, placenta, mRNA Partial, 2403 nt] SEQ ID NO: 511 201696 INCYTE INCYTE SEQ ID NO: 512 202259 INCYTE INCYTE SEQ ID NO: 513 2024815 g35496 H.sapiens mRNA for protein kinase C gamma (partial). SEQ ID NO: 514 203852 g1177434 H.sapiens mRNA for unknown 14 kDa protein. SEQ ID NO: 515 203960 g189675 Human vacuolar H+ ATPase proton channel subunit mRNA, complete cds. SEQ ID NO: 516 204502 INCYTE INCYTE SEQ ID NO: 517 2045226 INCYTE INCYTE SEQ ID NO: 518 2048834 g2253262 Rattus norvegicus neuronal pentraxin receptor mRNA, complete cds SEQ ID NO: 519 205155 g2244605 Human gene for TMEM1 and PWP2, complete a SEQ ID NO: 520 2059533 g183802 Human alpha-globin gene cluster on chromosome 16, pseudogene psi-a2 SEQ ID NO: 521 206130 INCYTE INCYTE SEQ ID NO: 522 2062218 g2224541 KIAA0300 SEQ ID NO: 523 206465 INCYTE INCYTE SEQ ID NO: 524 206520 g50003 Mouse mRNA for adipocyte p27 protein. SEQ ID NO: 525 206587 INCYTE INCYTE SEQ ID NO: 526 206638 INCYTE INCYTE SEQ ID NO: 527 207052 g2370071 Human DNA sequence from PAC 204E5 on chromosome 12. Contains exon SEQ ID NO: 528 207681 g1730287 Human acetolactate synthase homolog mRNA, complete cds. SEQ ID NO: 529 2079250 g1226237 Mus musculus cytochrome P450 Cyp7b1 mRNA, complete cds SEQ ID NO: 530 2100016 g313837 A.thaliana gene for hemC. SEQ ID NO: 53i 212088 g1845344 Human placental equilibrative nucleoside transporter 1 (hENT1) mRNA,

complete cds. SEQ ID NO: 532 2130869 INCYTE INCYTE SEQ ID NO: 533 213940 INCYTE INCYTE SEQ ID NO: 534 215150 g644878 Human Gps1 (GPS1) mRNA, complete cds. SEQ ID NO: 535 2160348 g431321 EC 3.4.16; cleaves C-terminal amino acids linked to penultimate proline; prolylcarboxypeptidase SEQ ID NO: 536 216982 INCYTE INCYTE SEQ ID NO: 537 216991 INCYTE INCYTE SEQ ID NO: 538 021786 g1099250 Homo sapiens cDNA clone 231999 3' SEQ ID NO: 539 2186852 g24762 H.sapiens mRNA fragment for alpha-2 macroglobulin receptor. SEQ ID NO: 540 2192167 g1150648 EC 2.4.1.83; dolichyl-phosphate- mannose synthase SEQ ID NO: 541 2198554 g306811 glutathione S-transferase SEQ ID NO: 542 2203436 g1679778 Human nucleosome assembly protein 2 mRNA, complete cds. SEQ ID NO: 543 221877 g1336022 Human HeLa mRNA isolated as a false positive in a two-hybrid-screen. SEQ ID NO: 544 2219639 g2463647 Mus musculus snRNP core Sm protein homolog Sm-X5 (Sm-X5) gene, two SEQ ID NO: 545 2220010 g1508382 H.sapiens flow-sorted chromosome 6 HindIII fragment, SC6pA22F2. SEQ ID NO: 546 2223685 g510281 Human mRNA for kinesin-related protein, partial cds. SEQ ID NO: 547 222689 g189569 Human plasminogen activator inhibitor 1 (PAI-1) gene, exon 2. SEQ ID NO: 548 224798 g832913 Human high molecular weight B cell growth factor mRNA sequence. SEQ ID NO: 549 2252906 g303602 Human mRNA for cytochrome P-450LTBV. SEQ ID NO: 550 2256528 g206619 Rat 55 RNA gene, clone 5S-6 SEQ ID NO: 551 2258960 PubEST PubEST SEQ ID NO: 552 2259319 g1552995 Human erythroid-specific transcription factor EKLF mRNA, complete cds. SEQ ID NO: 553 2270581 g248 CI-B9; EC 1.6.99; NADH dehydrogenase SEQ ID NO: 554 2271485 g36556 H.sapiens Sox-8 mRNA. SEQ ID NO: 555 2279032 g2317645 Homo sapiens mRNA for smallest subunit of ubiquinol-cytochrome c reductase, complete cds. SEQ ID NO: 556 2284186 g699497 IkB beta SEQ ID NO: 557 2315951 g1301622 C08B6 SEQ ID NO: 558 2349047 g829619 Fas interacting protein; cell death; RIP SEQ ID NO: 559 2353627 g191228 Hamster uridine diphosphate N-acetyl D-glucosamine dolichol phosphate N-acetyl-glucosamine-1 phosphatetransferase mRNA SEQ ID NO: 560 2356044 g1429348 NHP2; high-mobility-group protein SEQ ID NO: 561 2365149 g1161342 Mouse interleukin 17 receptor mRNA, comp SEQ ID NO: 562 236660 INCYTE INCYTE SEQ ID NO: 563 237704 INCYTE INCYTE SEQ ID NO: 564 239988 INCYTE INCYTE SEQ ID NO: 565 240885 INCYTE Homo sapiens interleukin 9 receptor (IL9R) gene, complete cds. SEQ ID NO: 566 2448372 g1550782 M.musculus mRNA for transcription factor BARX1. 2448372 SEQ ID NO: 567 2471348 g951301 M.musculus GEG-154 mRNA. SEQ ID NO: 568 2473119 g307437 Human pre-mRNA splicing factor SRp75 mRNA, complete cds. SEQ ID NO: 569 255361 INCYTE INCYTE SEQ ID NO: 570 257321 INCYTE INCYTE SEQ ID NO: 571 263518 g55820 R.norvegicus mRNA for brain-derived neurotrophic factor (exon IV). SEQ ID NO: 572 264226 INCYTE INCYTE SEQ ID NO: 573 270483 g1854034 Human Cdc5-related protein (PCDC5RP) mRNA, complete cds. SEQ ID NO: 574 274605 INCYTE INCYTE SEQ ID NO: 575 275010 g1753108 Human cyclin A1 mRNA, complete cds. SEQ ID NO: 576 2804907 g866469 Homo sapiens cDNA clone 150936 5' similar to contains Alu repetitive element SEQ ID NO: 576 2804907 g292359 Human NFG genomic fragment. SEQ ID NO: 577 285202 g165652 protein kinase delta SEQ ID NO: 578 287240 g1009451 S.pombe chromosome I cosmid c22G7 SEQ ID NO: 579 287586 INCYTE INCYTE SEQ ID NO: 580 288492 g1291337 Soares fetal lung NbHL19W Homo sapiens cDNA clone 301455 5' similar to WP:W06B4.2 CE02891 SEQ ID NO: 581 289171 INCYTE INCYTE SEQ ID NO: 582 290214 INCYTE INCYTE SEQ ID NO: 583 290510 g1665778 Human mRNA for KIAA0256 gene, complete cds. SEQ ID NO: 584 290628 INCYTE INCYTE SEQ ID NO: 585 291736 INCYTE INCYTE SEQ ID NO: 586 2918759 g338630 Human synaptobrevin 2 (SYB2) gene, exon 5. SEQ ID NO: 587 2922560 INCYTE INCYTE SEQ ID NO: 588 029244 g756234 Homo sapiens cDNA clone 125197 5' similar to gb:M14565 CYTOCHROME P450 XIA1, MITOCHONDRIAL (HUMAN) SEQ ID NO: 589 292708 INCYTE

INCYTE SEQ ID NO: 590 3038216 g1469884 KIAA0151 SEQ ID NO: 591 3043265 PubEST PubEST SEQ ID NO: 592 3044325 g788133 Homo sapiens cDNA clone 134940 5' similar to contains Alu repetitive element SEQ ID NO: 593 309389 INCYTE INCYTE SEQ ID NO: 594 3100562 g516680 Chicken gene for c-maf proto- oncogene product c-Maf, short form yv87e05 SEQ ID NO: 595 310202 g2415582 Homo sapiens mRNA for Marenostrin protein, complete. SEQ ID NO: 596 310487 g33942G Human T cell-specific protein (RANTES) mRNA, complete cds. SEQ ID NO: 597 3125445 g2224588 Human mRNA for KIAA0324 gene, partial cds. SEQ ID NO: 598 318358 g2224600 Human mRNA for KIAA0330 gene, partial cds. SEQ ID NO: 599 318438 INCYTE INCYTE SEQ ID NO: 600 318444 INCYTE INCYTE SEQ ID NO: 601 318774 g1064915 H.sapiens mRNA for ubiquitin conjugating enzyme, UbcH7. SEQ ID NO: 602 3188122 g2266637 Human OB-RGRP gene. SEQ ID NO: 603 3191066 g2280475 Human mRNA for KIAA0315 gene, partial cds. SEQ ID NO: 604 319684 INCYTE INCYTE SEQ ID NO: 605 320014 g1558796 Homo sapiens CDNA clone 525535 5' similar to SPA1 MOUSE P46062 GTPASE-ACTIVATING PROTEIN **SPA-1** SEQ ID NO: 606 320811 INCYTE INCYTE SEQ ID NO: 607 321651 INCYTE INCYTE SEQ ID NO: 608 334959 INCYTE INCYTE SEQ ID NO: 609 335100 INCYTE INCYTE SEQ ID NO: 610 336724 INCYTE INCYTE SEQ ID NO: 611 338196 INCYTE INCYTE SEQ ID NO: 612 338339 INCYTE INCYTE SEQ ID NO: 613 338345 INCYTE INCYTE SEQ ID NO: 614 338368 INCYTE INCYTE SEQ ID NO: 615 338435 INCYTE INCYTE SEQ ID NO: 616 339045 INCYTE INCYTE SEQ ID NO: 617 339198 INCYTE INCYTE SEQ ID NO: 618 339335 INCYTE INCYTE SEQ ID NO: 619 339678 INCYTE INCYTE SEQ ID NO: 620 339997 INCYTE INCYTE SEQ ID NO: 621 340100 INCYTE INCYTE SEQ ID NO: 622 340318 INCYTE INCYTE SEQ ID NO: 623 340422 INCYTE INCYTE SEQ ID NO: 624 340450 INCYTE INCYTE SEQ ID NO: 625 340883 INCYTE INCYTE SEQ ID NO: 626 341595 INCYTE INCYTE SEQ ID NO: 627 342342 g806765 Human 76 kDa tyrosine phosphoprotein SLP-76 mRNA, complete cds. SEQ ID NO: 628 343466 INCYTE INCYTE SEQ ID NO: 629 343595 g1184698 Human tyrosyl-tRNA synthetase mRNA, complete cds SEQ ID NO: 630 343619 BL00425A Arthropod defensins proteins. SEQ ID NO: 631 344012 INCYTE INCYTE SEQ ID NO: 632 345315 INCYTE INCYTE SEQ ID NO: 633 345380 g337810 Human MAR/SAR DNA binding protein (SATB1) mRNA, complete cds. SEQ ID NO: 634 345409 INCYTE INCYTE SEQ ID NO: 635 345472 INCYTE INCYTE SEQ ID NO: 636 346439 INCYTE INCYTE SEQ ID NO: 637 346597 g8651 Mst87F; structural sperm protein SEQ ID NO: 638 346869 g2257694 Homo sapiens mRNA for SCGF, complete cds. SEQ ID NO: 639 347184 g1197073 GEF1 SEQ ID NO: 640 003490 g30337 Human CYP2D7BP pseudogene for cytochrome SEQ ID NO: 641 349715 INCYTE INCYTE SEQ ID NO: 642 3518373 g2282039 Homo sapiens Arp2/3 protein complex subunit p20-Arc (ARC20) mRNA SEQ ID NO: 643 3523611 g1710211 Human clone 23732 mRNA, partial cds SEQ ID NO: 644 3534074 g19867 extensin (AA 1-620) SEQ ID NO: 645 3538629 g1386895 Soares fetal heart NbHH19W Homo sapiens cDNA clone 345320 5' simiiar to SWLCOGY_MOUSE Q02853 STROMELYSIN-3 PRECURSOR SEQ ID NO: 646 358673
g2465410 Homo sapiens Bcl-1/Bcl-2 binding protein (BAD) mRNA, partial cds SEQ ID NO: 647 361577 g189389 Homo sapiens osteogenic protein-2 (OP-2) mRNA, complete cds. SEQ ID NO: 648 369126 g1905905 Homo sapiens DNA from chromosome 19p13.2 cosmids R31240, R30272 and R28549 containing the EKLF, GCDH, CRTC, and RAD23A genes, genomic sequence. SEQ ID NO: 649 375230 g2505956 Rattus norvegicus mRNA for 70 kDa tumor specific antigen, partial.

	L #	Hits	Search T xt	DBs	Time Stamp
1	L1	436	ikk$2	USPAT; US-PGPUB	2003/11/04 09:50
2	L2	584058	complex	USPAT; US-PGPUB	2003/11/04 09:50
3	L3	120	1 same 2	USPAT; US-PGPUB	2003/11/04 09:51
4	L4	25	spa-1	USPAT; US-PGPUB	2003/11/04 14:41
5	L5	3	1 and 4	USPAT; US-PGPUB	2003/11/04 14:41
6	L6	6	4 same 2	USPAT; US-PGPUB	2003/11/04 14:43

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ABSTRACT:

Methods are provided for labeling nucleic acid molecules for use in hybridization reactions, and kits employing these methods. The level of labeling is increased by including one or more reactive modifications, such as amine-modifications, into the primers used to initiate synthesis of the nucleic acid molecule, for instance through random-primed reverse transcription. Also provided are modified random primers (such as amine-modified random primers) useful in these methods, labeling and hybridization kits comprising such primers, labeled nucleic acid molecules and mixtures of molecules, and methods for using them. Methods are also provided for amplifying a nucleic acid template contained within extremely small samples, in some cases as little as one cell. In particular embodiments, a single random primer is used for all steps of the amplification method. The nucleic acid template can either be of cellular or viral origin.

REFERENCE TO RELATED APPLICATIONS

[0001] This is a continuation in part of International Patent Application No. PCT/US02/11656, filed Apr. 11, 2002, which in turn claims the benefit of U.S. Provisional Application No. 60/283,423, filed Apr. 11, 2001, both of which are incorporated herein by reference.

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Detail Description Table CWU - DETL (6):

6TABLE 3 GenBank No Unigene No dTa dTb dTc P2a P2b P2c Clone description AI849214 Mm.105330 18.3218 17.5265 16.2183 20.7580 25.5790 22.7692 whey acidic protein AI848293 Mm.34507 6.9711 5.3423 3.5613 8.1450 6.4472 6.7642 ESTs AI847098 Mm.29982 5.2438 4.5541 4.5709 5.6932 5.1469 5.7707 ERO1-like (S. cerevisiae) AI852317 Mm.4063 3.7839 3.5895 4.4260 5.4368 5.0830 5.2313 N-myc downstream regulated 1 AI844828 Mm.2834 3.7150 3.7105 3.9967 5.1164 4.9883 4.7269 glycine transporter 1 AI846827 Mm.70667 5.2250 4.0641 3.4577 4.6474 4.2576 4.2443 Mus musculus, Similar to oxidation resistance 1 AI843085 Mm.157648 5.5280 4.4538 4.7526 4.5177 4.0203 4.3967 RIKEN cDNA 5730403B10 gene AI842716 Mm.140158 5.5015 5.8588 5.1314 4.4732 4.4336 4.4295 cytochrome P450, 51 AI836864 Mm.4704 6.6261 5.7066 3.7317 4.4534 3.9560 4.4178 forkhead box G1 AI853347 Mm.21884 4.0523 3.9847 3.3449 4.4364 4.7968 4.3672 ESTs, Weakly similar to GTPase-activating protein **SPA-1** AI843677 Mm.45357 3.7376 3.5943 3.5423 3.8309 3.4541 3.3920 Erbb2 interacting protein AI838612 Mm.14601 3.0926 3.3974 3.2623 3.6027 3.4499 3.4159 glutathione S-transferase, mu 2 AI848205 Mm.35844 3.6669 3.3875 3.1423 3.4911 3.0100 3.1019 growth arrest specific 5 AI850589 Mm.22627 3.7784 3.1037 3.1616 3.2339 3.2407 3.6818 epidermal growth factor receptor pathway substrate 15 AI852765 Mm.24193 0.3300 0.3343 0.3183 0.3254 0.2847 0.3249 glypican 1 AI836264 Mm.4871 0.1492 0.1253 0.1183 0.3200 0.3100 0.2357 tissue inhibitor of metalloproteinase 3 AI844851 Mm.10406 0.3209 0.3235 0.2910 0.3243 0.2993 0.3025 RIKEN cDNA 3110001M13 gene AI851985 Mm.29586 0.2668 0.2559 0.2278 0.3233 0.2814 0.3107 RIKEN cDNA 2610024P12 gene AI845475 Mm.30811 0.1031 0.1333 0.1210 0.3180 0.3200 0.3100 ESTs AI853172 Mm.27173 0.2968 0.3133 0.3032 0.3132 0.2847 0.3100 ectoplacental cone sequence AI835858 Mm.27685 0.2834 0.2925 0.2512 0.3114 0.3067 0.2751 ESTs, Highly similar to tropomyosin 4 [Rattus norvegicus] AI836045 Mm.29976 0.2461 0.3202 0.2812 0.3016 0.3236 0.2702 septin 5 AI843823 Mm.7414 0.1481 0.1690 0.1445 0.2971 0.3129 0.2507 neuron specific gene family member 1 AI844342 Mm.182255 0.1773 0.2039 0.2446 0.2833 0.3164 0.3083 CD97 antigen AI835331 Mm.544 0.2802 0.3336 0.3057 0.2829 0.1995 0.2646 phosphoprotein enriched in astrocytes 15 AI845602 Mm.4146 0.2438 0.2668 0.3188 0.2727 0.2349 0.2469 platelet derived growth factor receptor, beta polypeptide AI838302 Mm.4426 0.2816 0.2966 0.3223 0.2702 0.2466 0.2872 Cd63 antigen AI835546 Mm.3117 0.2023 0.2238 0.2903 0.2696 0.3022 0.3240 T-cell death associated gene AI853531 Mm.21679 0.2340 0.3006 0.3272 0.2691 0.2573 0.2708 RIKEN cDNA 1300002F13 gene AI842302 Mm.4139 0.3176 0.3029 0.3261 0.2652 0.2259 0.2783 rhotekin AI835620 No Data 0.2793 0.3169 0.3180 0.2637 0.2298 0.2679 No Data AI845774 Mm.856 0.2799 0.2757 0.3172 0.2630 0.2362 0.2575 transmembrane 4 superfamily member 1 AI838659 Mm.262 0.2496 0.2866 0.3001 0.2484 0.2192 0.2592 ras homolog gene family, member C AI848618 Mm.29010 0.1939 0.2150 0.2075 0.2473 0.2205 0.2216 membrane bound C2 domain containing protein AI851997 Mm.29010 0.2759 0.2851 0.3298 0.2462 0.2379 0.2648 membrane bound C2 domain containing protein AI852812 Mm.2308 0.2209 0.2669 0.3063 0.2409 0.2236 0.2485 hemoglobin Z, beta-like embryonic chain AI844356 Mm.1017 0.2547 0.2658 0.2582 0.2261 0.2191 0.2255 esterase 10 AI851647 Mm.22240 0.2365 0.2571 0.2440 0.2219 0.2185 0.2236 ESTs, Weakly similar to SH3BGR protein AI838551 Mm.2792 0.1605 0.1832

0.1807 0.2191 0.1398 0.2238 prostaglandin- endoperoxide synthase 1 AI842654 Mm.8180 0.2336 0.2595 0.2941 0.2182 0.2249 0.2627 lymphocyte antigen 6 **complex** AI841122 Mm.39804 0.2427 0.2581 0.3048 0.2139 0.2408 0.2015 EST AI838653 Mm.181074 0.2615 0.2885 0.3198 0.2073 0.2179 0.2407 RIKEN cDNA 2610001E17 gene AI838959 Mm.16537 0.1483 0.1504 0.2370 0.2014 0.2943 0.2463 actin, alpha 2, smooth muscle, aorta AI842847 Mm.8245 0.2013 0.2803 0.2512 0.1975 0.1770 0.1926 tissue inhibitor of metalloproteinase AI838351 No Data 0.1422 0.1998 0.0999 0.1913 0.3317 0.2076 No Data AI837390 Mm.43278 0.1418 0.1444 0.1499 0.1882 0.2873 0.2535 olfactomedin related ER localized protein AI844326 Mm.194675 0.2317 0.2675 0.2290 0.1847 0.0958 0.1462 EST AI839057 No Data 0.2107 0.2988 0.2685 0.1806 0.2179 0.2184 No Data AI838085 Mm.687 0.1668 0.1773 0.2450 0.1781 0.2298 0.2301 aplysia ras-related homolog B (RhoB) AI837494 Mm.39836 0.1604 0.1709 0.2824 0.1768 0.1658 0.1247 ESTs, Weakly similar to T14318 ubiquitin- protein ligase E3- alpha AI836532 Mm.196484 0.1481 0.1464 0.1405 0.1645 0.1642 0.1756 EST AA408841 AI835609 Mm.1956 0.0364 0.0776 0.0791 0.1608 0.2416 0.1599 neurofilament, light polypeptide AI842984 Mm.980 0.1258 0.1350 0.1376 0.1602 0.2456 0.1732 tenascin C AI849378 Mm.2769 0.1639 0.1670 0.1944 0.1545 0.1712 0.2004 MARCKS-like protein AI839275 Mm.738 0.1356 0.1868 0.2704 0.1503 0.2651 0.1883 procollagen, type IV, alpha 1 AI844626 Mm.29975 0.0684 0.1024 0.1284 0.1489 0.1956 0.1716 RIKEN cDNA 1810003P21 gene AI835201 Mm.8739 0.1115 0.1536 0.1402 0.1454 0.1709 0.1867 sarcoglycan, epsilon AI844312 Mm.3091 0.1443 0.2400 0.2183 0.1432 0.2094 0.1778 epsin 1 AI841755 Mm.687 0.1340 0.1510 0.1345 0.1427 0.1610 0.1485 aplysia ras-related homolog B (RhoB) AI838813 Mm.192516 0.1338 0.1664 0.1652 0.1416 0.1249 0.1655 EST AI839735 Mm.37751 0.1409 0.1558 0.1463 0.1403 0.1138 0.1486 ESTs AI837031 Mm.157662 0.0520 0.0994 0.1407 0.1260 0.0776 0.0931 synaptotagmin 13 AI840673 Mm.29924 0.0846 0.0945 0.1128 0.1237 0.1111 0.1437 ADP-ribosylation-like factor 6 interacting protein AI841538 Mm.41009 0.1166 0.1329 0.2839 0.1210 0.1168 0.1004 Nedd4 WW-binding protein 4 AI847958 Mm.20246 0.1447 0.1526 0.2049 0.1173 0.0934 0.1017 RIKEN cDNA 2410004D18 gene AI840633 Mm.38021 0.0477 0.1194 0.1215 0.1122 0.0823 0.0391 carbohydrate (keratan sulfate Gal-6) sulfotransferase 1 AI843323 Mm.3900 0.1334 0.1957 0.2642 0.1120 0.0902 0.1358 latent transforming growth factor beta binding protein 2 AI849869 Mm.34113 0.1241 0.1336 0.1955 0.1120 0.1015 0.1198 VPS10 domain receptor protein SORCS 2 AI840335 Mm.39154 0.0928 0.1347 0.1007 0.1104 0.1833 0.1133 EST AI840972 Mm.29580 0.2618 0.3083 0.3024 0.1059 0.1794 0.1681 superiorcervical ganglia, neural specific 10 AI847162 Mm.29357 0.0973 0.0696 0.2018 0.1050 0.1264 0.1312 RIKEN cDNA 1300017C10 gene AI843174 Mm.29924 0.1284 0.1426 0.1479 0.1044 0.1134 0.1473 ADP-ribosylation-like factor 6 interacting protein AI839366 Mm.28947 0.0651 0.1159 0.1742 0.1021 0.1278 0.1395 ESTs AI840692 No Data 0.1394 0.1457 0.1741 0.0917 0.1456 0.1644 No Data AI835703 Mm.29975 0.0961 0.0827 0.0714 0.0868 0.1302 0.1381 RIKEN cDNA 1810003P21 gene AI836865 Mm.44102 0.0503 0.0643 0.1129 0.0842 0.1727 0.1572 ESTs AI842983 Mm.192586 0.0702 0.1325 0.1346 0.0785 0.1555 0.1091 EST AI839950 Mm.3126 0.0492 0.0610 0.0989 0.0781 0.2076 0.1304 four and a half LIM domains 1 AI844604 Mm.3126 0.1263 0.1328 0.1465 0.0750 0.0188 0.0613 four and a half LIM domains 1 AI836826 Mm.2976 0.0747 0.0764 0.0755 0.0747 0.0918 0.0759 glycoprotein 38 AI850497 Mm.41072 0.1133 0.1862 0.2509 0.0743 0.1009 0.0891 ESTs, Highly similar to LOX5 mouse arachidonate 5- lipoxygenase AI835403 Mm.142729 0.0965 0.1012 0.1014 0.0620 0.0778 0.0579 thymosin, beta 4, X chromosome AI848096 Mm.17951 0.1483 0.1711 0.1888 0.0580 0.1324 0.1233 erythrocyte protein band 4.1-like 3 AI843282 Mm.181021 0.0955 0.1120 0.1453 0.0529 0.0995 0.1095 procollagen, type IV,

alpha 2 AI842554 Mm.192583 0.0577 0.0889 0.0962 0.0428 0.1088 0.0815 ESTs AI842681 Mm.20904 0.0702 0.0488 0.1056 0.0405 0.0375 0.0487 cartilage associated protein AI835976 Mm.17951 0.0491 0.0372 0.0362 0.0392 0.0591 0.0431 erythrocyte protein

US-PAT-NO: 6607879

DOCUMENT-IDENTIFIER: US 6607879 B1

TITLE: Compositions for the detection of blood cell and immunological response gene expression

DATE-ISSUED: August 19, 2003

INVENTOR-INFORMATION:

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Stuart; Susan G.	Montara	CA	N/A	N/A
Seilhamer; Jeffrey J.	Los Altos Hills	CA	N/A	N/A

APPL-NO: 09/ 023655

DATE FILED: February 9, 1998

US-CL-CURRENT: 435/6, 435/69.1 , 536/23.1 , 536/24.1 , 536/24.3 , 536/24.31 , 536/24.32 , 536/24.33

ABSTRACT:

The present invention relates to a composition comprising a plurality of polynucleotide probes. The composition can be used as hybridizable array elements in a microarray. The present invention also relates to a method for selecting polynucleotide probes for the composition.

7 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

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Detailed Description Paragraph Table - DETL (5):

SEQ ID NO: 492 198126 INCYTE INCYTE SEQ ID NO: 493 199094 INCYTE INCYTE SEQ ID NO: 494 199150 INCYTE INCYTE SEQ ID NO: 495 199173 INCYTE INCYTE SEQ ID NO: 496 199305 INCYTE INCYTE SEQ ID NO: 497 199690 g1184317 Human inhibitor of apoptosis protein 2 mRNA, complete cds. SEQ ID NO: 498 199812 INCYTE INCYTE SEQ ID NO: 499 1999147 g1041680 Rattus norvegicus phospholipase A-2-activating protein (plap) mRNA, complete cds. SEQ ID NO: 500 200015 INCYTE INCYTE SEQ ID NO: 501 200044 g1136395 Human mRNA for KIAA0168 gene, complete cds. gb100pri SEQ ID NO: 502 200097 INCYTE INCYTE SEQ ID NO: 503 200212 INCYTE INCYTE SEQ ID NO: 504 2006402 g1216374 Rat Tclone4 mRNA. SEQ ID NO: 505 200844 INCYTE INCYTE SEQ ID NO: 506 201349 INCYTE INCYTE SEQ ID NO: 507

201358 INCYTE INCYTE SEQ ID NO: 508 201392 BL00257 Bombesin-like peptides family proteins. SEQ ID NO: 509 201507 INCYTE INCYTE SEQ ID NO: 510 2016903 g247306 cytochrome P450 reductase [human, placenta, mRNA Partial, 2403 nt] SEQ ID NO: 511 201696 INCYTE INCYTE SEQ ID NO: 512 202259 INCYTE INCYTE SEQ ID NO: 513 2024815 g35496 H.sapiens mRNA for protein kinase C gamma (partial). SEQ ID NO: 514 203852 g1177434 H.sapiens mRNA for unknown 14 kDa protein. SEQ ID NO: 515 203960 g189675 Human vacuolar H+ ATPase proton channel subunit mRNA, complete cds. SEQ ID NO: 516 204502 INCYTE INCYTE SEQ ID NO: 517 2045226 INCYTE INCYTE SEQ ID NO: 518 2048834 g2253262 Rattus norvegicus neuronal pentraxin receptor mRNA, complete cds SEQ ID NO: 519 205155 g2244605 Human gene for TMEM1 and PWP2, complete a SEQ ID NO: 520 2059533 g183802 Human alpha-globin gene cluster on chromosome 16, pseudogene psi-a2 SEQ ID NO: 521 206130 INCYTE INCYTE SEQ ID NO: 522 2062218 g2224541 KIAA0300 SEQ ID NO: 523 206465 INCYTE INCYTE SEQ ID NO: 524 206520 g50003 Mouse mRNA for adipocyte p27 protein. SEQ ID NO: 525 206587 INCYTE INCYTE SEQ ID NO: 526 206638 INCYTE INCYTE SEQ ID NO: 527 207052 g2370071 Human DNA sequence from PAC 204E5 on chromosome 12. Contains exon SEQ ID NO: 528 207681 g1730287 Human acetolactate synthase homolog mRNA, complete cds. SEQ ID NO: 529 2079250 g1226237 Mus musculus cytochrome P450 Cyp7b1 mRNA, complete cds SEQ ID NO: 530 2100016 g313837 A.thaliana gene for hemC. SEQ ID NO: 53i 212088 g1845344 Human placental equilibrative nucleoside transporter 1 (hENT1) mRNA, complete cds. SEQ ID NO: 532 2130869 INCYTE INCYTE SEQ ID NO: 533 213940 INCYTE INCYTE SEQ ID NO: 534 215150 g644878 Human Gps1 (GPS1) mRNA, complete cds. SEQ ID NO: 535 2160348 g431321 EC 3.4.16; cleaves C-terminal amino acids linked to penultimate proline; prolylcarboxypeptidase SEQ ID NO: 536 216982 INCYTE INCYTE SEQ ID NO: 537 216991 INCYTE INCYTE SEQ ID NO: 538 021786 g1099250 Homo sapiens cDNA clone 231999 3' SEQ ID NO: 539 2186852 g24762 H.sapiens mRNA fragment for alpha-2 macroglobulin receptor. SEQ ID NO: 540 2192167 g1150648 EC 2.4.1.83; dolichyl-phosphate- mannose synthase SEQ ID NO: 541 2198554 g306811 glutathione S-transferase SEQ ID NO: 542 2203436 g1679778 Human nucleosome assembly protein 2 mRNA, complete cds. SEQ ID NO: 543 221877 g1336022 Human HeLa mRNA isolated as a false positive in a two-hybrid-screen. SEQ ID NO: 544 2219639 g2463647 Mus musculus snRNP core Sm protein homolog Sm-X5 (Sm-X5) gene, two SEQ ID NO: 545 2220010 g1508382 H.sapiens flow-sorted chromosome 6 HindIII fragment, SC6pA22F2. SEQ ID NO: 546 2223685 g510281 Human mRNA for kinesin-related protein, partial cds. SEQ ID NO: 547 222689 g189569 Human plasminogen activator inhibitor 1 (PAI-1) gene, exon 2. SEQ ID NO: 548 224798 g832913 Human high molecular weight B cell growth factor mRNA sequence. SEQ ID NO: 549 2252906 g303602 Human mRNA for cytochrome P-450LTBV. SEQ ID NO: 550 2256528 g206619 Rat 55 RNA gene, clone 5S-6 SEQ ID NO: 551 2258960 PubEST PubEST SEQ ID NO: 552 2259319 g1552995 Human erythroid-specific transcription factor EKLF mRNA, complete cds. SEQ ID NO: 553 2270581 g248 CI-B9; EC 1.6.99; NADH dehydrogenase SEQ ID NO: 554 2271485 g36556 H.sapiens Sox-8 mRNA. SEQ ID NO: 555 2279032 g2317645 Homo sapiens mRNA for smallest subunit of ubiquinol-cytochrome c reductase, complete cds. SEQ ID NO: 556 2284186 g699497 IkB beta SEQ ID NO: 557 2315951 g1301622 C08B6 SEQ ID NO: 558 2349047 g829619 Fas interacting protein; cell death; RIP SEQ ID NO: 559 2353627 g191228 Hamster uridine diphosphate N-acetyl D-glucosamine dolichol phosphate N-acetyl-glucosamine-1 phosphatetransferase mRNA SEQ ID NO: 560 2356044 g1429348 NHP2; high-mobility-group protein SEQ ID NO: 561 2365149 g1161342 Mouse interleukin 17 receptor mRNA, comp SEQ ID NO: 562 236660 INCYTE INCYTE SEQ ID NO: 563 237704 INCYTE INCYTE SEQ ID NO: 564 239988 INCYTE INCYTE SEQ ID NO: 565 240885 INCYTE Homo sapiens interleukin 9 receptor

(IL9R) gene, complete cds. SEQ ID NO: 566 2448372 g1550782 M.musculus mRNA for transcription factor BARX1. 2448372 SEQ ID NO: 567 2471348 g951301 M.musculus GEG-154 mRNA. SEQ ID NO: 568 2473119 g307437 Human pre-mRNA splicing factor SRp75 mRNA, complete cds. SEQ ID NO: 569 255361 INCYTE INCYTE SEQ ID NO: 570 257321 INCYTE INCYTE SEQ ID NO: 571 263518 g55820 R.norvegicus mRNA for brain-derived neurotrophic factor (exon IV). SEQ ID NO: 572 264226 INCYTE INCYTE SEQ ID NO: 573 270483 g1854034 Human Cdc5-related protein (PCDC5RP) mRNA, complete cds. SEQ ID NO: 574 274605 INCYTE INCYTE SEQ ID NO: 575 275010 g1753108 Human cyclin A1 mRNA, complete cds. SEQ ID NO: 576 2804907 g866469 Homo sapiens cDNA clone 150936 5' similar to contains Alu repetitive element SEQ ID NO: 576 2804907 g292359 Human NFG genomic fragment. SEQ ID NO: 577 285202 g165652 protein kinase delta SEQ ID NO: 578 287240 g1009451 S.pombe chromosome I cosmid c22G7 SEQ ID NO: 579 287586 INCYTE INCYTE SEQ ID NO: 580 288492 g1291337 Soares fetal lung NbHL19W Homo sapiens cDNA clone 301455 5' similar to WP:W06B4.2 CE02891 SEQ ID NO: 581 289171 INCYTE INCYTE SEQ ID NO: 582 290214 INCYTE INCYTE SEQ ID NO: 583 290510 g1665778 Human mRNA for KIAA0256 gene, complete cds. SEQ ID NO: 584 290628 INCYTE INCYTE SEQ ID NO: 585 291736 INCYTE INCYTE SEQ ID NO: 586 2918759 g338630 Human synaptobrevin 2 (SYB2) gene, exon 5. SEQ ID NO: 587 2922560 INCYTE INCYTE SEQ ID NO: 588 029244 g756234 Homo sapiens cDNA clone 125197 5' similar to gb:M14565 CYTOCHROME P450 XIA1, MITOCHONDRIAL (HUMAN) SEQ ID NO: 589 292708 INCYTE INCYTE SEQ ID NO: 590 3038216 g1469884 KIAA0151 SEQ ID NO: 591 3043265 PubEST PubEST SEQ ID NO: 592 3044325 g788133 Homo sapiens cDNA clone 134940 5' similar to contains Alu repetitive element SEQ ID NO: 593 309389 INCYTE INCYTE SEQ ID NO: 594 3100562 g516680 Chicken gene for c-maf proto- oncogene product c-Maf, short form yv87e05 SEQ ID NO: 595 310202 g2415582 Homo sapiens mRNA for Marenostrin protein, complete. SEQ ID NO: 596 310487 g33942G Human T cell-specific protein (RANTES) mRNA, complete cds. SEQ ID NO: 597 3125445 g2224588 Human mRNA for KIAA0324 gene, partial cds. SEQ ID NO: 598 318358 g2224600 Human mRNA for KIAA0330 gene, partial cds. SEQ ID NO: 599 318438 INCYTE INCYTE SEQ ID NO: 600 318444 INCYTE INCYTE SEQ ID NO: 601 318774 g1064915 H.sapiens mRNA for ubiquitin conjugating enzyme, UbcH7. SEQ ID NO: 602 3188122 g2266637 Human OB-RGRP gene. SEQ ID NO: 603 3191066 g2280475 Human mRNA for KIAA0315 gene, partial cds. SEQ ID NO: 604 319684 INCYTE INCYTE SEQ ID NO: 605 320014 g1558796 Homo sapiens CDNA clone 525535 5' similar to SPA1 MOUSE P46062 GTPASE-ACTIVATING PROTEIN **SPA-1** SEQ ID NO: 606 320811 INCYTE INCYTE SEQ ID NO: 607 321651 INCYTE INCYTE SEQ ID NO: 608 334959 INCYTE INCYTE SEQ ID NO: 609 335100 INCYTE INCYTE SEQ ID NO: 610 336724 INCYTE INCYTE SEQ ID NO: 611 338196 INCYTE INCYTE SEQ ID NO: 612 338339 INCYTE INCYTE SEQ ID NO: 613 338345 INCYTE INCYTE SEQ ID NO: 614 338368 INCYTE INCYTE SEQ ID NO: 615 338435 INCYTE INCYTE SEQ ID NO: 616 339045 INCYTE INCYTE SEQ ID NO: 617 339198 INCYTE INCYTE SEQ ID NO: 618 339335 INCYTE INCYTE SEQ ID NO: 619 339678 INCYTE INCYTE SEQ ID NO: 620 339997 INCYTE INCYTE SEQ ID NO: 621 340100 INCYTE INCYTE SEQ ID NO: 622 340318 INCYTE INCYTE SEQ ID NO: 623 340422 INCYTE INCYTE SEQ ID NO: 624 340450 INCYTE INCYTE SEQ ID NO: 625 340883 INCYTE INCYTE SEQ ID NO: 626 341595 INCYTE INCYTE SEQ ID NO: 627 342342 g806765 Human 76 kDa tyrosine phosphoprotein SLP-76 mRNA, complete cds. SEQ ID NO: 628 343466 INCYTE INCYTE SEQ ID NO: 629 343595 g1184698 Human tyrosyl-tRNA synthetase mRNA, complete cds SEQ ID NO: 630 343619 BL00425A Arthropod defensins proteins. SEQ ID NO: 631 344012 INCYTE INCYTE SEQ ID NO: 632 345315 INCYTE INCYTE SEQ ID NO: 633 345380 g337810 Human MAR/SAR DNA binding protein (SATB1) mRNA, complete cds. SEQ ID NO: 634 345409 INCYTE INCYTE SEQ ID NO: 635 345472 INCYTE INCYTE SEQ ID NO: 636

346439 INCYTE INCYTE SEQ ID NO: 637 346597 g8651 Mst87F; structural sperm protein SEQ ID NO: 638 346869 g2257694 Homo sapiens mRNA for SCGF, complete cds. SEQ ID NO: 639 347184 g1197073 GEF1 SEQ ID NO: 640 003490 g30337 Human CYP2D7BP pseudogene for cytochrome SEQ ID NO: 641 349715 INCYTE INCYTE SEQ ID NO: 642 3518373 g2282039 Homo sapiens Arp2/3 protein **complex** subunit p20-Arc (ARC20) mRNA SEQ ID NO: 643 3523611 g1710211 Human clone 23732 mRNA, partial cds SEQ ID NO: 644 3534074 g19867 extensin (AA 1-620) SEQ ID NO: 645 3538629 g1386895 Soares fetal heart NbHH19W Homo sapiens cDNA clone 345320 5' simiiar to SWLCOGY_MOUSE Q02853 STROMELYSIN-3 PRECURSOR SEQ ID NO: 646 358673
g2465410 Homo sapiens Bcl-1/Bcl-2 binding protein (BAD) mRNA, partial cds SEQ ID NO: 647 361577 g189389 Homo sapiens osteogenic protein-2 (OP-2) mRNA, complete cds. SEQ ID NO: 648 369126 g1905905 Homo sapiens DNA from chromosome 19p13.2 cosmids R31240, R30272 and R28549 containing the EKLF, GCDH, CRTC, and RAD23A genes, genomic sequence. SEQ ID NO: 649 375230 g2505956 Rattus norvegicus mRNA for 70 kDa tumor specific antigen, partial.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	436	ikk$2	USPAT; US-PGPUB	2003/11/04 09:50
2	L2	584058	complex	USPAT; US-PGPUB	2003/11/04 09:50
3	L3	120	1 same 2	USPAT; US-PGPUB	2003/11/04 09:51
4	L4	25	spa-1	USPAT; US-PGPUB	2003/11/04 14:41
5	L5	3	1 and 4	USPAT; US-PGPUB	2003/11/04 14:41
6	L6	6	4 same 2	USPAT; US-PGPUB	2003/11/04 14:43
7	L7	2341	nfkb or (nf adj kappa adj b) or (nf adj kb)	USPAT; US-PGPUB	2003/11/04 14:45
8	L8	2	6 and 7	USPAT; US-PGPUB	2003/11/04 14:46
9	L9	7	4 and 7	USPAT; US-PGPUB	2003/11/04 14:46

PGPUB-DOCUMENT-NUMBER: 20030157526

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030157526 A1

TITLE: Identification of genetic markers of biological age and metabolism

PUBLICATION-DATE: August 21, 2003

INVENTOR-INFORMATION:

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Prolla, Tomas A.	Madison	WI	US	
Lee, Cheol-Koo	Madison	WI	US	

APPL-NO: 10/ 307706

DATE FILED: December 2, 2002

RELATED-US-APPL-DATA:

child 10307706 A1 20021202

parent division-of 09630567 20000808 US PENDING

non-provisional-of-provisional 60148540 19990812 US

non-provisional-of-provisional 60178232 20000126 US

non-provisional-of-provisional 60211923 20000616 US

US-CL-CURRENT: 435/6

ABSTRACT:

A method of measuring the biological age of a multicellular organism is disclosed. In one embodiment this method comprises the steps of obtaining a sample of nucleic acid isolated from the organism's organ, tissue or cell and determining the expression pattern of a panel of sequences within the nucleic acid that have been predetermined by either increase or decrease in response to biological aging of the organ, tissue or cell. A method of obtaining biomarkers of aging is also disclosed. This method comprises the step of comparing a gene expression profile of a young multicellular organism subject's organ, tissue or cells; a gene expression profile from a chronologically aged subject's organ, tissue or cell; and a gene expression profile from a chronologically aged but biologically younger subject's organ, tissue or cell and identifying gene expression alterations that are observed when comparing the young subjects and the chronologically aged subjects and are not observed or reduced in magnitude when comparing the young subjects and the

chronologically aged but biologically younger subjects.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to provisional application 60/148,540, filed Aug. 12, 1999, U.S. provisional application 60/178,232, filed Jan. 26, 2000 and 60/211,923 filed Jun. 16, 2000. These provisional applications are incorporated by reference as if fully set forth herein.

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Detail Description Table CWU - DETL (8):

8TABLE 8 Caloric restriction-related decreases in gene expression in neocortex of C57BL/6 mice* CR De- Signal Intensity ORF crease SE CR Control Gene Class X76505 -7.2 1.0 -195 73 Tyro 10 Signal transduction U43088 -6.3 1.1 -109 164 IL-17 (CTLA-8) Immune/inflammatory W50186 -5.6 2.1 -38 129 Heavy chain homolog Unknown Y07711 -3.5 0.5 28 151 Zyxin Signal transduction Z47205 -3.1 0.8 45 200 PLZF Transcriptional factor AA000203 -2.8 0.7 -93 26 Corticosteroid-binding globulin precursor Transport W83658 -2.6 0.5 51 197 Guanine nucleotide-binding protein Signal transduction G(I)/G(S)/G(O) homolog L46815 -2.6 0.2 8 67 Ig kappa chain recombination and transcription DNA metabolism enhancer AA153484 -2.4 0.5 208 456 SERCA2 Ion transport W51466 -2.4 0.4 12 147 Chlorine channel protein P64 homolog Unknown U27398 -2.4 0.4 39 132 XPC DNA Metabolism X58069 -2.2 0.7 54 164 H2A.X DNA metabolism U50712 -2.2 0.4 54 156 MCP-5 Immune/inflammatory M61909 -2.1 0.3 39 125 **NF-kappa-B** p65 Stress response AA072643 -2.1 0.4 49 110 Midkine precursor homolog Stress response L01991 -2.1 0.3 48 132 PANG Unknown L04678 -2.1 0.2 -64 138 Integrin beta 4 subunit Structural W64628 -2.1 0.4 62 197 Guanine nucleotide-binding protein Signal transduction G(I)/G(S)/G(O) gamma-7 subunit X54098 -2.0 0.3 55 136 lamin B2 Structural AA023458 -2.0 0.3 20 107 Heat shock 27 KD protein homolog Stress response D63380 -2.0 0.2 -19 32 Alpha-1,3-fucosyltransferase Protein metabolism U15548 -2.0 0.3 -30 42 Beta 2 thyroid hormone receptor Energy metabolism AA123385 -2.0 0.2 57 117 Phosphorylase B kinase gamma catalytic chain Energy metabolism X57349 -2.0 0.4 -10 49 Transferrin receptor Transport D00659 -2.0 0.1 1 35 Aromatase P450 Biosynthesis AA028875 -2.0 0.2 -32 54 Glycine-rich cell wall structural homolog Lysosomal X76291 -2.0 0.1 11 79 Ihh (Indian Hedgehog) Signal transduction AA041982 -1.9 0.3 44 84 LARK Circadian regulation AA118758 -1.9 0.2 103 206 Multifunctional aminoacyl-tRNA synthetase Protein synthesis W75353 -1.9 0.3 90 162 Apolipoprotein C-IV Transport W55410 -1.9 0.2 30 111 Tubulin gamma chain homolog Unknown L20343 -1.9 0.2 22 102 L-type calcium channel beta 2a subunit isoform Transport W91095 -1.9 0.5 44 93 Valyl-tRNA synthetase Protein metabolism X81593 -1.9 0.1 53 119 Winged-helix domain Transcriptional factor M38248 -1.9 0.2 -6 25 BALB8N Unknown J04694 -1.8 0.3 48 134 Alpha-1 type IV collagen Structural L47650 -1.8 0.3 50 85 STAT6 R Immune/inflammatory AA023595 -1.8 0.1 38 133 Frizzled protein precursor Signal transduction AA015168 -1.8 0.2 42 97 Interferon-gamma receptor beta chain homolog Immune/inflammatory AA013951 -1.8 0.1 32 38 Creatine transporter homolog Energy metabolism W78443 -1.8 0.2 17 106 MKP-X Signal transduction D31842 -1.8 0.2 66 126 PTP36 Structural W50138 -1.8 0.2 1 162 Putative serine/threonine-protein kinase B0464.5 Unknown L35307 -1.8 0.2 33 104 c-Krox

Transcriptional factor AA073154 -1.8 0.3 31 68 Alpha-catern homolog Structural W12720 -1.8 0.3 149 251 RAP-2B homolog Signal transduction AA170169 -1.8 0.2 -17 37 Elongation factor 1-gamma homolog Protein metabolism W48951 -1.8 0.3 8 30 Voltage-dependent anion-selective channel Unknown protein 2 homolog M35732 -1.8 0.3 -13 17 Seminal vesicle secretory protein IV Unknown AA145515 -1.8 0.3 68 187 Pre-MRNA splicing factor PRP6 RNA metabolism W13162 -1.8 0.1 -7 62 Cell division protein kinase 4 DNA metabolism J03482 -1.8 0.2 42 113 Histone H1 DNA metabolism W82793 -1.8 0.1 -4 59 Topoisomerase E III homolog DNA metabolism Z31360 -1.8 0.3 1 51 P/L01 Unknown Y09632 -1.8 0.1 16 37 Rabkinesin-6 Transport AA066621 -1.8 0.2 13 63 60S ribosomal protein L10 Protein metabolism U67874 -1.8 0.3 46 85 Ubiqurtin thiolesterase family Protein metabolism AA109714 -1.8 0.3 562 968 SKP1 RNA metabolism AA007957 -1.8 0.2 210 357 Threonyl-tRNA synthetase homolog Protein metabolism AA162633 -1.8 0.2 46 95 Isoleucyl-tRNA synthetase Protein metabolism M17299 -1.8 0.3 29 101 Phosphoglycerate kinase (pgk-2) Energy metabolism AA050102 -1.7 0.3 211 263 Elongation factor 2 (EF-2) Protein metabolism W54637 -1.7 0.2 72 137 Tubulin bets-2 chain class-II homolog Unknown D10028 -1.7 0.3 167 312 Glutamate receptor channel subunit zeta 1 Neurotransmission M28587 -1.7 0.2 -52 30 Alpha leukocyte interferon Immune/inflammatory AA023506 -1.7 0.2 60 144 Insulin receptor substrate-3 Energy metabolism W70629 -1.7 0.3 92 158 COPII Protein metabolism U33626 -1.7 0.3 66 125 PML isoform 1 (Pml) Unknown AA144746 -1.7 0.2 42 92 EF-1-delta Protein metabolism M19380 -1.7 0.3 1406 2303 Calmodulin (Cam III) Signal transduction AA144136 -1.7 0.2 43 100 Choline kinase R1 homolog Biosynthesis AA165847 -1.7 0.3 331 509 EF-1-alpha2 homolog Protein metabolism W33415 -1.7 0.2 90 136 ATP citrate-lyase Unknown U35233 -1.6 0.1 71 109 Endothelin-1 Vasoconstrictive peptide W57384 -1.9 0.3 6 15 ATP synthase A chain homolog Energy metabolism X60452 -1.6 0.3 124 200 Cytochrome P-450IIIA Stress response AA022127 -1.6 0.1 172 279 Vascular endothelial growth factor Unknown AA168841 -1.6 0.2 169 289 Serine/threonine-protein kinase PAK Unknown AA120586 -1.6 0.1 9 64 Apolipoprotein B-100 precursor Stress response AA104561 -1.6 0.2 104 166 EIF-4A homolog Protein metabolism X17071 -1.6 0.1 25 90 Trophoblast-specific protein Growth factor M96265 -1.6 0.1 153 250 Galactose-1-phosphate uridyl transferase Biosynthesis AA145160 -1.6 0.2 178 287 Translational initiation factor 2 alpha Protein metabolism X63473 -1.6 0.1 69 110 m4 muscannic acetylcholine receptor Neurotransmission AA002750 -1.5 0.2 176 290 5-lipoxygenase activating protein (FLAP) Immune/inflammatory W64698 -1.5 0.2 51 63 Protein kinase C inhibitor 1 Signal transduction U63841 -1.5 0.1 120 197 NeuroD3 Growth factors U04294 -1.5 0.1 99 150 Potassium channel subunit (m-eag) Transport M33227 -1.5 0.2 259 396 Cryptdin-related (CRS4C) Immune/ inflammatory U20532 -1.5 0.1 45 67 P45 NF-E2 related factor 2 (Nrf2) Transcriptional factor AA140026 -1.5 0.1 378 519 DNA directed RNA polymerase polypeptide G DNA metabolism W09025 -1.5 0.1 47 68 ATP synthase B chain homolog Energy metabolism W29163 -1.5 0.1 342 465 Leydig cell tumor 10kd protein homolog Unknown AA155191 -1.5 0.1 36 65 Kinesin heavy chain Transport M80360 -1.5 0.1 63 96 Rep-3 DNA metabolism AA044561 -1.4 0.2 93 132 PEP carboxykinase - mitochondnal Energy metabolism AA096843 -1.4 0.2 130 175 Unknown Unknown X57277 -1.4 0.1 908 1298 Rac 1 Signal transduction W82998 -1.4 0.1 256 363 BUB3 DNA metabolism *The values presented for Signal Intensity are the averages at three mice per age group and are expressed as data for old CR/old control mice. The SE was calculated for the nine pairwise comparisons and was obtained by dividing the standard deviation by the square root of 3. The method from which signal intensity is used to estimate fold

changes is described in the Methods section of the manuscript.

Detail Description Table CWU - DETL (9):

9TABLE 9 Aging-related increases in gene expression in the cereum of C57BL/6 mice* Fold Signal Intensity CR ORF Change SE Old Young Gene Class Prevention AA120109 9.3 3.4 254 29 Interferon-induced protein 6-16 precursor Immune/inflammatory N M21050 6.4 0.9 291 14 Lysozyme P (Lzp-s) Immune 88 X56824 5.7 1.9 160 89 Tumor-induced 32 kD protein (p32) Unknown 100 V00727 5.6 2.6 282 57 c-fos Stress 30 M13019 4.9 0.7 109 3 Thymidylale synthase DNA metabolism 87 L16894 4.7 1.0 192 5 Cyclophilin C (CyCAP) Immune/inflammatory N AA146437 4.7 0.3 841 169 Cathepsin S precursor Stress 62 X58861 4.4 0.2 719 160 C1Q alpha-chain Immune/inflammatory 80 W67046 4.3 0.8 50 1 C6 chemokine Immune/inflammatory N X66295 4.1 0.6 508 147 C1q C-chain Immune/inflammatory 56 W65899 4.1 1.8 152 58 Guanine nucleotide-binding protein Signal transduction 80 U00677 4.1 2.2 16 -10 Syntrophin-1 Neurotransmission 100 X68273 3.9 1.8 108 -37 Macrosialin Immune/inflammatory N U19854 3.9 0.5 35 -63 Ubiqurtinating enzyme E2-20K Protein metabolism 100 U63133 3.9 1.1 318 95 Emv-3 Viral N L20315 3.8 0.1 97 26 MPS1 Immune/inflammatory 56 K01347 3.8 0.7 337 109 Glial fibrillary acidic protein (GFAP) Stress 61 M17440 3.7 0.3 445 116 Sex-limited protein (SlpA) Immune/inflammatory N X91144 3.6 1.3 38 -2 P-selectin glycoprotein ligand 1 Immune/inflammatory 100 U43084 3.5 0.8 54 18 IFIT-2 Glucocorticoid-attenuated response Immune/inflammatory N AA089333 3.4 0.2 208 61 Cathepsin S precursor Stress 71 X83733 3.4 0.3 71 -7 SAP62-AMH RNA metabolism 100 W45750 3.3 1.3 197 257 Guanine nucleotide-binding protein G(T) Signal transduction 100 M22531 3.3 0.2 431 146 Clq B-chain Immune/inflammatory 65 AA031244 3.1 0.4 83 9 DNAJ protein homolog HSJ1 Stress 100 M60429 3.1 0.8 121 37 Ig-gamma 1 chain Immune/inflammatory 100 AA036067 3.0 0.4 815 311 Apolipoprotein E precursor (APO-E) Lipid transport 28 U06119 2.9 0.3 27 4 Cathepsin H prepropeptide (ctsH) Stress response 55 AA106347 2.9 0.3 243 57 Angiotensinogen precursor Osmoregulation 80 W98998 2.9 0.7 182 79 Neurogenic locus notch homolog protein 1 Immune/inflammatory 100 AA059700 2.8 0.3 2013 687 MHC class I B(2)-microglobulin Immune/inflammatory 45 U73037 2.8 0.8 69 41 Interferon regulatory factor 7 (7) Immune/inflammatory 50 Y00964 2.8 0.3 780 316 beta-hexosaminidase (Hexb) Unknown 47 X55315 2.8 0.6 63 15 Fetus cerebral cortex for 3UTR Transcription factor 100 U37465 2.8 0.1 15 -7 Protein tyrosine phosphatase phi (PTPphi) Unknown 63 L07803 2.7 1.2 24 -15 trombospondin 2 Structural N U19119 2.7 0.3 52 -5 G-proiein-like LRG-47 Immune/inflammatory N X52886 2.6 0.2 893 326 Cathepsin D Stress response 38 W70578 2.6 1.2 31 7 Antigen WC1 1 Immune/inflammatory 81 X16705 2.6 0.4 93 -4 Laminin B1 Structural 84 W57539 2.6 0.3 28 6 Oocyte zinc finger protein XLCOF8 Unknown N X52308 2.6 0.4 32 9 Thrombin Fibrinogen activation 91 U70859 2.6 0.7 109 46 Cationic amino acid transporter (CAT3) AA transport 49 U41497 2.6 1.1 160 40 Very-long chain acyl-CoA dehydrogenase Lipid metabolism 100 AA089339 2.6 0.5 76 31 Cystatin C precursor Immune/inflammatory 100 X16151 2.5 0.1 239 95 Early T-lymphocyte activation 1 protein Immune/inflammatory 49 U37419 2.5 0.5 111 -2 G protein alpha subunit (GNA-15) Unknown N K02785 2.5 0.5 15 -6 r-fos Stress response N M12289 2.5 0.5 39 25 Pennatal skeletal myosin heavy chain Structural 100 X58849 2.4 0.4 59 13 Murine Hox-4.7 Developmental 100 AA063858 2.4 0.2 89 32 Rho-related GTP-binding protein RHOG Signal transduction 74 D10632 2.4 0.2 33 -27 Zinc finger protein Transcription factor N U33005 2.3 0.4 35 -8 tbc1 Unknown N

W85160 2.3 0.7 70 41 40S ribosomal protein S4, X isoform Unknown 100 U57331 2.3 1.0 42 15 Transcription factor Tbx6 (tbx6) Developmental 92 U44731 2.3 0.2 71 20 Putative purine nucleotide binding protein Immune/inflammatory N W87253 2.3 0.6 58 16 Integrin beta-5 Subunit precursor Cell adhesion 100 U53142 2.3 0.2 223 101 Endothelial constitutive nitric oxide Synthase Neurotranmission N AA087715 2.3 0.1 85 -6 GTPase-activating protein **SPA-1** Unknown N D49429 2.3 0.3 554 251 Rad21 homolog DNA metabolism 73 AA155318 2.3 0.4 291 129 HNRP1 RNA metabolism N AA032593 2.3 0.1 99 17 Transducin beta chain 2 Signal transduction 83 X03690 2.3 0.2 45 -13 Ig mu chain Immune/inflammatory 93 M26417 2.3 0.5 54 28 T cell receptor beta chain Immune/inflammatory 100 X86374 2.2 0.6 73 38 TAG7 Immune/inflammatory 38 W90894 2.2 0.3 27 -11 Cell division protein kinase 4 DNA metabolism 100 M84005 2.2 0.7 83 51 Olfactory receptor 15 Odor receptor 23 X55573 2.2 0.5 55 19 Brain-derived neurotrophic factor Growth factor N W30129 2.2 0.3 90 -16 Phosphatidylinositol glycan hmolog Structural 100 AA163771 2.2 0.3 153 67 EIF-28 epsilon subunit Protein metabolism N X72910 2.1 0.4 96 44 HSA-C Unknown N AA116604 2.1 0.2 303 181 Cathepsin Z Stress response 64 L16462 2.1 0.4 51 4 BCL2-related protein A1 Apoptosis 58 L13732 2.1 0.4 53 29 Natl. resistance-asstd. macrophage protein1 Immune/inflammatory 85 D37791 2.1 0.1 934 424 Beta-1,4-galactosyltransferase Protein metabolism 82 AA125097 2.0 0.1 618 313 Unknown Unknown 94 AA109998 2.0 0.2 40 12 Hexokinase D homolog Energy metabolism 100 M88127 2.0 0.2 33 -8 APC2 homolog Unknown 82 X13538 2.0 0.5 114 45 Hox-1,4 Growth/development 100 V01527 2.0 0.5 28 10 H2-IA-beta Immune/inflammatory 100 AA144411 2.0 0.1 86 79 Unknown Unknown 100 X63535 2.0 0.1 55 21 Tyrosine-protein kinase receptor UFO Signal transduction N M83348 2.0 0.1 42 22 Pregnancy specific glycoprotein homolog Unknown N W08211 2.0 0.2 62 26 TGF-beta receptor type III Signal transduction 100 W13136 2.0 0.4 266 87 Angiotenisinogen Osmoregulation 36 W46084 2.0 0.1 89 45 Unknown Unknown N U73744 2.0 0.1 3958 2909 Heat shock 70 Stress response 100 D29763 1.9 0.2 465 271 Seizure-related, product 6 type 3 Unknown 47 AA118121 1.9 1.0 51 37 Isoleucyl-tRNA synthetase Protein metabolism N M27034 1.9 0.2 258 163 MHC class 1 D-region Immune/inflammatory N U35249 1.9 0.1 68 36 CDK-activating kinase assembly factor DNA metabolism 61 J03776 1.9 0.4 37 22 Down regulatory protein (rpt-1r) of IL-2 receptor Immune/inflammatory N U28728 1.9 0.3 221 112 Els Signal transduction 66 AA124192 1.9 0.2 411 244 Unknown Unknown 44 W63809 1.8 0.4 136 80 Unknown Unknown 73 X16834 1.8 0.2 455 182 Galectin-3 Immune/inflammatory N X16995 1.8 0.2 351 221 N10 nuclear hormonal receptor homolog Unknown 100 J02870 1.8 0.2 848 380 40S ribosomal protein SA Protein metabolism 100 L21768 1.8 0.2 153 76 EGF15 Growth factor 68 AA117284 1.8 0.1 217 123 Zinc finger protein homolog Unknown N *The values presented for Signal Intensity are the averages of three mice per age group and are expressed as data for old/young mice. The prevention by CR is shown as being none (N) or the calculated percentage effect The SE was calculated for the nine pairwise compansons and was obtained by dividing the standard deviation by the square root of 3. The method from which signal intensity is used to estimate fold changes is described in the Methods section of the manuscript.

Detail Description Table CWU - DETL (10):

10TABLE 10 Aging-related increases in gene expression in the cereum of C57BL/6 mice* Fold Signal Intensity CR ORF Change SE Old Young Gene Class Prevention U00445 -4.3 1.4 39 132 Glucose-6-phosphatase Energy metabolism 79 W48504 -4.1 1.1 32 78 phosphoneuroprotein 14 homolog) Unknown N AA153337 -3.9

0.7 67 218 Myosin regulatory light chain 2 (MLC-2). Unknown 61 W51213 -3.9 0.5 14 57 NEDD-4 homolog Protein metabolism 55 X56304 -3.1 0.4 2 27 Tenascin Growth/development N W12681 -3.1 0.6 30 126 Hepatocyte growth factor Growth/development 37 Z68889 -2.9 1.0 30 70 Wnt-2 homolog Growth/development N W55684 -2.8 0.6 13 37 Brain protein i47 Unknown N U04827 -2.8 0.5 94 219 Brain fatty acid-binding protein (B-FABP) Growth/development N AA008066 -2.7 1.0 1 61 Pre-mRNA splicing factor PRP22 Unknown 74 W55300 -2.7 0.7 20 47 Fatty acid-binding protein, heart (H-FABP) Unknown 71 D13903 -2.7 0.5 7 37 MPTPdelta (type A) Growth/development N AA013976 -2.6 0.5 162 405 POL polyprotein; reverse transcriptase; Unknown N ribonuclease H W10865 -2.6 0.2 14 142 Myosin light chain 1, atnal/foetal isoform Unknown N AA020296 -2.5 0.2 -162 166 NG9 Growth/development 100 W64865 -2.5 1.1 10 31 Stat-3 Unknown N AA139694 -2.5 0.3 64 203 Beta-myosin heavy chain Transport 100 U29762 -2.5 0.3 304 657 Albumin gene D-Box binding protein Transcription Factor N M87276 -2.4 0.5 16 34 Thrombospondin Structural 52 X02677 -2.4 0.2 63 160 Anion exchange protein Anion exchanger 100 X04836 -2.4 0.2 22 68 T-cell antigen CD4 Immune/inflammatory 100 X87242 -2.4 0.3 48 111 unc-33 Growth/development 70 AA163021 -2.4 0.2 28 143 Annexin VIII Signal transduction 84 M31810 -2.4 0.3 29 113 P-protein membrane transporter Transport 100 M97900 -2.4 0.6 18 49 Unknown Unknown 20 M15008 -2.4 0.6 101 227 Steroid 21-hydroxylase B Steroid metabolism 100 M99377 -2.4 0.5 77 191 Alpha-2 adrenergic receptor Neurotransmission N M32490 -2.4 0.3 62 122 Cyr61 Growth/development 41 AA168350 -2.3 0.3 130 237 Cysteinyl-tRNA synthetase Protein metabolism 83 AA061206 -2.3 0.2 8 52 Unp (ubiquitin protease) Protein metabolism N W12794 -2.3 0.3 23 96 Unknown Unknown 78 AA050593 -2.3 0.1 5 69 Unknown Unknown 62 AA050715 -2.3 0.3 64 148 Smoothelin Structural 92 AA106463 -2.2 0.3 110 277 Phosphoenolpyruvate carboxykinase. Energy metabolism N X90829 -2.2 0.3 -16 9 Lbx1 Growth/development N X65588 -2.2 0.3 -1 24 mp41 Neurotransmission N J00475 -2.2 0.2 -23 58 Ig alpha chain Immune/inflammatory N X03019 -2.2 0.3 4 71 GM-CSF Immune/inflammatory 26 W34687 -2.2 0.4 62 115 Alpha-actin Transport 78 W75614 -2.2 0.4 27 56 Alpha-synuclein Growth/development N AA068153 -2.2 0.3 14 39 Polyadenylate-binding protein RNA metabolism 55 U36842 -2.1 0.5 22 36 Riap 3-inhibitor of apoptosis Apoptosis 100 W09127 -2.1 0.3 3 85 60S ribosomal protein L22 Protein metabolism 100 D63819 -2.1 0.2 29 87 Neuropeptide Y-Y1 receptor Neurotransmission N M33884 -2.1 0.1 70 139 Env polyprotein Viral protein 55 AA144430 -2.1 0.3 64 156 **NF-KB** P100 inhibitory subunit Stress response 48 AA168554 -2.1 0.3 119 246 Unknown Unknown 85 U35730 -2.1 0.8 12 30 Jerky Unknown N M92649 -2.1 0.4 45 112 nitric oxide synthase Neurotransmission N D12907 -2.1 0.2 55 126 Serine protease inhibitor homologue Unknown 85 M17327 -2.1 0.2 234 566 Env polyprotein Viral protein 56 AA170444 -2.1 0.2 172 246 Ubiquitin-activating enzyme E1 Protein metabolism 100 W12658 -2.1 0.3 203 415 FKBP-rapamycin associated protein Unknown N AA123026 -2.1 0.3 60 116 REG 2 Unknown 100 W13125 -2.1 0.5 111 232 Phenylalanyl-tRNA synthetase beta chain Protein metabolism N AA103862 -2.1 0.4 53 143 Unknown Unknown N U21301 -2.1 0.6 30 62 c-mer tyrosine kinase receptor Signal transduction N W13586 -2.1 0.1 29 136 Myosin light chain 1 homolog Transport 100 W42217 -2.1 0.1 69 143 Ribosomal protein S20 Protein metabolism 100 AA153522 -2.1 0.4 95 191 Serine/threonine kinase Signal transduction 78 W30612 -2.0 0.1 70 160 Chloride intracellular channel 3 Transport 100 W11621 -2.0 0.4 78 138 Zinc finger protein 126 Unknown N X72805 -2.0 0.3 25 63 CD-1 histone H1t DNA metabolism N L08407 -2.0 0.3 38 117 Collagen type XVII Structural N AA145609 -2.0 0.2 55 134 cAMP responsive element modifier Transcriptional factor 34 W12756 -2.0 0.1 48 117 Unknown Unknown 92 W75523

-2.0 0.3 48 95 Vertebrate homolog of C. elegans Lin-7 type 2 Unknown N D85904 -1.9 0.3 69 129 Heat shock 70-related protein Apg-2 Stress response N AA138911 -1.8 0.2 176 311 RNA helicase PRP16 RNA metabolism 100 W42216 -1.8 0.1 183 361 SWI/SNF related homolog Transcriptional factor 74 W12395 -1.8 0.4 141 237 Transcription elongation factor A (SII) Transcriptional factor 88 K03235 -1.8 0.1 84 149 Prolifenn 2 Growth factor 100 AA145859 -1.8 0.1 4110 5250 Unknown Unknown 100 W57194 -1.8 0.2 61 108 Ubiquitin carboxyl terminal hydrolase 12 Protein metabolism N AA166440 -1.7 0.1 229 389 Phosphatidylserine decarboxylase Protein metabolism N L33726 -1.7 0.1 69 128 Fascin homolog 1 Structural 100 L35549 -1.7 0.4 30 38 Y-box binding protein homolog Unknown 100 AA154514 -1.7 0.1 7639 12878 ATP synthase A chain (protein 6) homolog Energy metabolism 100 AA143937 -1.7 0.1 384 697 Beta-centractin Transport 70 AA027387 -1.7 0.1 169 270 Rab-4B Transport 51 L38971 -1.7 0.2 205 334 Integral membrane protein 2 Unknown 43 W10526 -1.7 0.1 193 301 Ca** channel, voltage-dep., gamma subunit 1 Transport 90 W12204 -1.6 0.2 114 200 Ca2+/calmodulin-dependent protein kinase Signal transduction N isoform gamma B AA170173 -1.6 0.1 149 289 NTT-73 Transport 100 M64403 -1.6 0.1 126 208 Cyclin D1 homolog DNA metabolism 100 W13191 -1.6 0.1 288 347 Thyroid hormone receptor alpha 2 Energy metabolism 87 U47543 -1.6 0.1 121 205 NGF1-A binding protein 2 (NAB2) Growth factor N D70848 -1.6 0.2 154 246 Zic2 (cerebellar zinc finger protein) Neural development 77 X56518 -1.6 0.3 106 164 Acetylcholinesterase Neurotransmission N AA144588 -1.6 0.2 233 368 Beta-adrenergic receptor kinase 2 homolog Neurotransmission 33 AA139828 -1.6 0.1 224 351 gonadotropin inducible transcription repressor-1 Unknown 100 homolog AA061170 -1.6 0.2 43 65 WW-domain oxidoreductase homolog Unknown N X58287 -1.6 0.3 84 153 mR-PTPu Signal transduction N L13129 -1.6 0.1 162 220 Annexin A7 Exocytosis 90 D85037 -1.6 0.1 50 77 Doc2beta Neruotransmission N U30823 -1.6 0.2 55 102 Myocyte enhancer factor-2A Transcriptional factor 33 W64791 -1.6 0.1 92 143 Galactokinase Energy metabolism N X52622 -1.6 0.1 274 377 IN Viral protein 100 AA063914 -1.5 0.1 175 267 Alpha-tubulin Transport 64 *The values presented for Signal Intensity are the averages of three mice per age group and are expressed as data for old/young mice. The prevention by CR is shown as being none (N) or the calculated percentage effect. The SE was calculated for the nine pairwise comparisons and was obtained by dividing the standard deviation by the square root of 3. The method from which signal intensity is used to estimate fold changes is described in the Methods section of the manuscript.

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ABSTRACT:

The present invention relates to novel cardiovascular system related polynucleotides and the polypeptides encoded by these polynucleotides herein collectively known as "cardiovascular system antigens," and the use of such cardiovascular system antigens for detecting disorders of the cardiovascular system, particularly the presence of cancer of cardiovascular system tissues and cancer metastases. More specifically, isolated cardiovascular system associated nucleic acid molecules are provided encoding novel cardiovascular system associated polypeptides. Novel cardiovascular system polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human cardiovascular system associated polynucleotides and/or polypeptides. The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders related to the cardiovascular system, including cancer of cardiovascular system tissues, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The present invention further relates to methods and/or compositions for inhibiting the production and function of the polypeptides of the present invention.

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Summary of Invention - Table CWU - BSTL (30):

4TABLE 2 SEQ Score/ Clone ID Contig ID Analysis PFam/NR Accession Percent NO : Z ID: NO : X Method PFam/NR Description Number Identity NT From NT To HAHEE05 928673 20 blastx.2 (AC005531) similar to gb.vertline.AAD04728.1.v-ertline. 100% 39 422 mouse homeodomain- interacting protein 1 HAHHE12 969107 24 blastx.14 N-RAP [Mus musculus] gi.vertline.2351568.vertline.gb.vertline.AAC5 76% 307 621 3323.1.vertline. 67% 139 333 54% 460 612 78% 667 762 35% 514 708 75% 673 759 59% 481 576 32% 526 708 37% 598 717 34% 541 705 40% 601 705 34% 547 675 27% 457 621 27% 514 708 85% 762 803 26% 619 756 31% 541 693 55% 703 769 29% 544 675 30% 460 585 38% 619 696 34% 598 693 38% 619 696 47% 526 588 29% 598 690 29% 607 717 66% 457 492 47% 526 576 75% 627 662 40% 526 585 33% 616 696 36% 649 705 23% 460 585 37% 637 708 37% 637 708 56% 526 573 39% 723 791 57% 532 573 50% 762 797 50% 762 797 28% 711 794 27% 592 702 26% 526 594 29% 720 800 30% 415 483 HELEA45 954371 49 blastx.14 exopolyphosphatase gi.vertline.147343.vertline.gb.vertline.AAA24 89% 86 169 [Escherichia coli] 415.1.vertline. 100% 66 98 HELHF07 949067 78 HMMER PFAM: PF00202 38.85 95 295 1.8 Aminotransferases class- III pyridoxal-phosphate blastx.14 4-aminobutyrate gi.vertline.1742132.vertline.dbj.vertline.BAA1 85% 83 295 aminotransferase (EC 4871.1.vertline. 92% 21 98 2.6.1.19) 1 1 45% 246 311 aminotransferase). 100% 1 18 [Escherichia coli] HEMEK19 574209 92 HMMER PFAM: TonB dependent PF00593 46.8 81 233 2.1.1 receptor C-terminal region HEMEU54 947801 94 blastx.14 samaphorin G [Mus gi.vertline.1418942.vertlin-e.emb.vertline.CAA 96% 11 325 musculus] 66398.1.vertline. 50% 122 280 42% 128 298 51% 122 238 54% 14 112 35% 68 235 33% 134 328 42% 29 112 30% 122 238 41% 343 429 44% 248 301 55% 302 328 62% 305 328 45% 296 328 62% 305 328 HHBEM7O 756949 107 HMMER PFAM: Core histones PF00125 13.08 144 215 1.8 H2A, H2B, H3 and H4 HHBHO63 906947 121 HMMER PFAM: Phorbol esters/ PF00130 2.21 188 217 1.8 diacylglycerol binding domain HHFBX77 959805 136 blastx.14 (AB012308) B2HC gi.vertline.4033608.vertline.dbj.ver- tline.BAA3 83% 13 366 [Anthocidaris crassispina] 5136.1.vertline. HHFCA64 720849 137 HMMER PFAM: Zinc-binding PF00099 3.2 287 267 1.8 metalloprotease domain HHFGN31 908508 177 HMMER PFAM:KRAB box PF01352 64.1 93 215 2.1.1 HHFHC02 920510 192 HMMER PFAM: Eukaryotic protein PF00069 13.11 118 183 1.8 kinase domain blastx.14 (AB023658) gi.vertline.4512334.vertline.dbj.vertline.BAA7 94% 70 183 Ca/calmodulin-dependent 5246.1.vertline. protein kinase 1 HHFJN02 918358 214 blastx.14 retrovirus-related reverse pir.vertline.B25313.vertline.GNLRL1.vertline. 53% 297 208 transcriptase pseudogene - 54% 390 286 slow loris HHFON19 910891 257 HMMER PFAM: Dual specificity PF00782 154.8 316 732 2.1.1 phosphatase, catalytic domain blastx.14 (AF143321) unknown gi.vertline.4929222.vertline.gb.vertline.AAD3 68% 298 825 [Homo sapiens] 3910.1.vertline.AF143321_1 HHFUC26 960331 267 HMMER PFAM: Src homology PF00018 3.21 343 375 1.8 domain 3 HMEGH46 887791 287 HMMER PFAM: C2 domain PF00168 12.81 10 78 1.8 HULAI37 708923 307 HMMER PFAM: Core histones PF00125 13.48 99 173 1.8 H2A, H2B, H3 and H4 HULFB76 767873 313 HMMER PFAM: HIT family PF01230 24.6 67 147 2.1.1 HUSIW10 963324 330 blastx.14 (AF098499) No definition gi.vertline.3786408.vertline.gb.vertline.AAC6 48% 234 320 line found [Caenorhabditis 7396.1.vertline. 41% 149 241 elegans] HUSYA63 928021 335 blastx.14 (AF116865) hedgehog- gi.vertline.4868122.vertline.gb.vertline.AAD3 88% 251 439 interacting protein [Mus 1172.1.vertline.AF116865_1 musculus] HUSZH03 922852 341 blastx.14 C06A6.3 gene product

gi.vertline.1086626.vertline.gb.vertline.AAA8 34% 367 633 [Caenorhabditis elegans] 2295.1.vertline. 61% 259 297 HUSYN11 943237 345 HMMER PFAM: Core histones PF00125 13.67 238 315 1.8 H2A, H2B, H3 and H4 blastx.2 (AL137556) hypothetical emb.vertline.CAB7O810.1.vertline. 67% 137 319 protein [Homo sapiens] 96% 240 320 96% 241 321 HUSIE95 967176 366 blastx.14 GS2NA [Homo sapiens] gi.vertline.805095.vertline.gb.vertline.AA- B81 56% 229 5 551.1.vertline. 53% 496 413 37% 121 11 31% 388 332 33% 484 431 HUSIE08 908574 368 blastx.14 (AB024005) KRAB- gi.vertline.4514561.vertline.dbj.ve- rtline.BAA7 70% 38 229 containing zinc-finger 5468.1.vertline. protein KRAZ2 [Mus musculus] HUSHL86 960355 369 blastx.14 (AF151805) CGI-47 gi.vertline.4929563.vertline.gb.vertline.- AAD3 96% 1142 882 protein [Homo sapiens] 4042.1.vertline.AF151805_1 100% 1413 1330 HUSFF03 924616 389 blastx.14 (AF033276) A kinase gi.vertline.2852701.vertline.gb.vertline.AA- C0 83% 266 535 anchor protein [Mus 2208.1.vertline. 47% 541 591 musculus] HHFLU06 857884 445 HMMER PFAM: Adenylate and PF00211 108.8 17 268 2.1.1 Guanylate cyclase catalytic domain HHFKX28 971102 453 blastx.14 Similarity to Yeast gi.vertline.3881836.vertline.emb.vertline.CAB 76% 858 619 LPG22P protein 01454.1.vertline. 65% 495 409 (TR:G1151240); 1 1 91% 617 546 cDNA EST EMBL:C10626 comes from this gene; cDNA EST EMBL:C10848 HHFJM64 958384 455 blastx.2 (AF026504) **SPA-1** like gb.vertline.AAB81526.1.vertline. 83% 3 287 protein p1294 [Rattus 43% 323 664 norvegicus] 29% 799 1266 28% 847 1344 HHFCH52 911570 503 blastx.14 INSERTIN=TENSIN sp.vertline.G256713.vertline.G256713 95% 15 77 HOMOLOG. HHBGJ53 909912 525 HMMER PFAM: PH domain PF00169 38.3 160 267 2.1.1 HHBGG10 963849 526 blastx.14 (AB011527) MEGF1 gi.vertline.3449286.vertline.dbj.vertline.BAA3 90% 98 3 [Rattus norvegicus] 2458.1.vertline. 75% 210 112 45% 210 151 41% 219 184 HHBEG80 951688 533 HMMER PFAM: Core histones PF00125 12.4 371 436 1.8 H2A, H2B, H3 and H4 HEMGL56 767669 538 HMMER PFAM: Filamin/ABP280 PF00630 84.1 45 209 2.1.1 repeat. HEMDX96 935963 543 blastx.14 (AF111170) unknown gi.vertline.4314286.vertline.gb.vertline.AAD1 79% 491 255 [Homo sapiens] 5563.1.vertline. HEMBT61 939957 550 HMMER PFAM: Eukaryotic protein PF00069 76.6 16 285 2.1.1 kinase domain blastx.2 (AD000092) hypothetical gb.vertline.AAB51171.1.vertline. 71% 13 441 human serine-threonine protein kinase R31240_1 [Homo sapiens] HELGY02 948302 557 blastx.2 Similar to sulfatase gb.vertline.AAA83618.1.vertline. 59% 383 523 [Caenorhabditis elegans] HELGW31 610003 558 HMMER PFAM: Cytocbrome C PF01578 216.5 672 1286 2.1.1 assembly protein blastx.2 (AE000309) heme gb.vertline.AAC75259.1.vertline. 100% 603 1337 exporter protein C [Escherichia coli] HELGW31 957568 622 HMMER PFAM: Cytochrome C PF01578 200.9 990 421 2.1.1 assembly protein

Detail Description Paragraph - DETX (365):

[1235] Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 34 and 35. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/**NF-KB**/EGR, GAS/**NF-KB**, I1-2/NFAT, or **NF-KB**/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

Detail Description Paragraph - DETX (402):

[1264] **NF-KB** (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, **NF-KB** regulates the expression of genes involved in immune cell activation, control of apoptosis (**NF- KB** appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

Detail Description Paragraph - DETX (403):

[1265] In non-stimulated conditions, **NF-KB** is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I-KB is phosphorylated and degraded, causing **NF-KB** to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by **NF-KB** include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Detail Description Paragraph - DETX (404):

[1266] Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the **NF-KB** promoter element are used to screen the supernatants produced in Example 30. Activators or inhibitors of **NF-KB** would be useful in treating, preventing, and/or diagnosing diseases. For example, inhibitors of **NF-KB** could be used to treat those diseases related to the acute or chronic activation of **NF-KB,** such as rheumatoid arthritis.

Detail Description Paragraph - DETX (405):

[1267] To construct a vector containing the **NF-KB** promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the **NF-KB** binding site (GGGGACTTTCCC) (SEQ ID NO: 8), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

Detail Description Paragraph - DETX (408):

[1270] Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this **NF-KB**/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Detail Description Paragraph - DETX (409):

[1271] In order to generate stable mammalian cell lines, the **NF-KB**/SV40/SEAP cassette is removed from the above **NF-KB**/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the **NF-KB**/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with SalI and NotI.

Detail Description Paragraph - DETX (410):

[1272] Once **NF-KB**/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 32. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 32. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

US-PAT-NO: 6607879

DOCUMENT-IDENTIFIER: US 6607879 B1

TITLE: Compositions for the detection of blood cell and immunological response gene expression

DATE-ISSUED: August 19, 2003

INVENTOR-INFORMATION:

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Seilhamer; Jeffrey J.	Los Altos Hills	CA	N/A	N/A

APPL-NO: 09/ 023655

DATE FILED: February 9, 1998

US-CL-CURRENT: 435/6, 435/69.1 , 536/23.1 , 536/24.1 , 536/24.3 , 536/24.31 , 536/24.32 , 536/24.33

ABSTRACT:

The present invention relates to a composition comprising a plurality of polynucleotide probes. The composition can be used as hybridizable array elements in a microarray. The present invention also relates to a method for selecting polynucleotide probes for the composition.

7 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

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Detailed Description Paragraph Table - DETL (5):
SEQ ID NO: 492 198126 INCYTE INCYTE SEQ ID NO: 493 199094 INCYTE INCYTE SEQ ID NO: 494 199150 INCYTE INCYTE SEQ ID NO: 495 199173 INCYTE INCYTE SEQ ID NO: 496 199305 INCYTE INCYTE SEQ ID NO: 497 199690 g1184317 Human inhibitor of apoptosis protein 2 mRNA, complete cds. SEQ ID NO: 498 199812 INCYTE INCYTE SEQ ID NO: 499 1999147 g1041680 Rattus norvegicus phospholipase A-2-activating protein (plap) mRNA, complete cds. SEQ ID NO: 500 200015 INCYTE INCYTE SEQ ID NO: 501 200044 g1136395 Human mRNA for KIAA0168 gene, complete cds. gb100pri SEQ ID NO: 502 200097 INCYTE INCYTE SEQ ID NO: 503 200212 INCYTE INCYTE SEQ ID NO: 504 2006402 g1216374 Rat Tclone4 mRNA. SEQ ID NO: 505 200844 INCYTE INCYTE SEQ ID NO: 506 201349 INCYTE INCYTE SEQ ID NO: 507

201358 INCYTE INCYTE SEQ ID NO: 508 201392 BL00257 Bombesin-like peptides family proteins. SEQ ID NO: 509 201507 INCYTE INCYTE SEQ ID NO: 510 2016903 g247306 cytochrome P450 reductase [human, placenta, mRNA Partial, 2403 nt] SEQ ID NO: 511 201696 INCYTE INCYTE SEQ ID NO: 512 202259 INCYTE INCYTE SEQ ID NO: 513 2024815 g35496 H.sapiens mRNA for protein kinase C gamma (partial). SEQ ID NO: 514 203852 g1177434 H.sapiens mRNA for unknown 14 kDa protein. SEQ ID NO: 515 203960 g189675 Human vacuolar H+ ATPase proton channel subunit mRNA, complete cds. SEQ ID NO: 516 204502 INCYTE INCYTE SEQ ID NO: 517 2045226 INCYTE INCYTE SEQ ID NO: 518 2048834 g2253262 Rattus norvegicus neuronal pentraxin receptor mRNA, complete cds SEQ ID NO: 519 205155 g2244605 Human gene for TMEM1 and PWP2, complete a SEQ ID NO: 520 2059533 g183802 Human alpha-globin gene cluster on chromosome 16, pseudogene psi-a2 SEQ ID NO: 521 206130 INCYTE INCYTE SEQ ID NO: 522 2062218 g2224541 KIAA0300 SEQ ID NO: 523 206465 INCYTE INCYTE SEQ ID NO: 524 206520 g50003 Mouse mRNA for adipocyte p27 protein. SEQ ID NO: 525 206587 INCYTE INCYTE SEQ ID NO: 526 206638 INCYTE INCYTE SEQ ID NO: 527 207052 g2370071 Human DNA sequence from PAC 204E5 on chromosome 12. Contains exon SEQ ID NO: 528 207681 g1730287 Human acetolactate synthase homolog mRNA, complete cds. SEQ ID NO: 529 2079250 g1226237 Mus musculus cytochrome P450 Cyp7b1 mRNA, complete cds SEQ ID NO: 530 2100016 g313837 A.thaliana gene for hemC. SEQ ID NO: 53i 212088 g1845344 Human placental equilibrative nucleoside transporter 1 (hENT1) mRNA, complete cds. SEQ ID NO: 532 2130869 INCYTE INCYTE SEQ ID NO: 533 213940 INCYTE INCYTE SEQ ID NO: 534 215150 g644878 Human Gps1 (GPS1) mRNA, complete cds. SEQ ID NO: 535 2160348 g431321 EC 3.4.16; cleaves C-terminal amino acids linked to penultimate proline; prolylcarboxypeptidase SEQ ID NO: 536 216982 INCYTE INCYTE SEQ ID NO: 537 216991 INCYTE INCYTE SEQ ID NO: 538 021786 g1099250 Homo sapiens cDNA clone 231999 3' SEQ ID NO: 539 2186852 g24762 H.sapiens mRNA fragment for alpha-2 macroglobulin receptor. SEQ ID NO: 540 2192167 g1150648 EC 2.4.1.83; dolichyl-phosphate- mannose synthase SEQ ID NO: 541 2198554 g306811 glutathione S-transferase SEQ ID NO: 542 2203436 g1679778 Human nucleosome assembly protein 2 mRNA, complete cds. SEQ ID NO: 543 221877 g1336022 Human HeLa mRNA isolated as a false positive in a two-hybrid-screen. SEQ ID NO: 544 2219639 g2463647 Mus musculus snRNP core Sm protein homolog Sm-X5 (Sm-X5) gene, two SEQ ID NO: 545 2220010 g1508382 H.sapiens flow-sorted chromosome 6 HindIII fragment, SC6pA22F2. SEQ ID NO: 546 2223685 g510281 Human mRNA for kinesin-related protein, partial cds. SEQ ID NO: 547 222689 g189569 Human plasminogen activator inhibitor 1 (PAI-1) gene, exon 2. SEQ ID NO: 548 224798 g832913 Human high molecular weight B cell growth factor mRNA sequence. SEQ ID NO: 549 2252906 g303602 Human mRNA for cytochrome P-450LTBV. SEQ ID NO: 550 2256528 g206619 Rat 55 RNA gene, clone 5S-6 SEQ ID NO: 551 2258960 PubEST PubEST SEQ ID NO: 552 2259319 g1552995 Human erythroid-specific transcription factor EKLF mRNA, complete cds. SEQ ID NO: 553 2270581 g248 CI-B9; EC 1.6.99; NADH dehydrogenase SEQ ID NO: 554 2271485 g36556 H.sapiens Sox-8 mRNA. SEQ ID NO: 555 2279032 g2317645 Homo sapiens mRNA for smallest subunit of ubiquinol-cytochrome c reductase, complete cds. SEQ ID NO: 556 2284186 g699497 IkB beta SEQ ID NO: 557 2315951 g1301622 C08B6 SEQ ID NO: 558 2349047 g829619 Fas interacting protein; cell death; RIP SEQ ID NO: 559 2353627 g191228 Hamster uridine diphosphate N-acetyl D-glucosamine dolichol phosphate N-acetyl-glucosamine-1 phosphatetransferase mRNA SEQ ID NO: 560 2356044 g1429348 NHP2; high-mobility-group protein SEQ ID NO: 561 2365149 g1161342 Mouse interleukin 17 receptor mRNA, comp SEQ ID NO: 562 236660 INCYTE INCYTE SEQ ID NO: 563 237704 INCYTE INCYTE SEQ ID NO: 564 239988 INCYTE INCYTE SEQ ID NO: 565 240885 INCYTE Homo sapiens interleukin 9 receptor

(IL9R) gene, complete cds. SEQ ID NO: 566 2448372 g1550782 M.musculus mRNA for transcription factor BARX1. 2448372 SEQ ID NO: 567 2471348 g951301 M.musculus GEG-154 mRNA. SEQ ID NO: 568 2473119 g307437 Human pre-mRNA splicing factor SRp75 mRNA, complete cds. SEQ ID NO: 569 255361 INCYTE INCYTE SEQ ID NO: 570 257321 INCYTE INCYTE SEQ ID NO: 571 263518 g55820 R.norvegicus mRNA for brain-derived neurotrophic factor (exon IV). SEQ ID NO: 572 264226 INCYTE INCYTE SEQ ID NO: 573 270483 g1854034 Human Cdc5-related protein (PCDC5RP) mRNA, complete cds. SEQ ID NO: 574 274605 INCYTE INCYTE SEQ ID NO: 575 275010 g1753108 Human cyclin A1 mRNA, complete cds. SEQ ID NO: 576 2804907 g866469 Homo sapiens cDNA clone 150936 5' similar to contains Alu repetitive element SEQ ID NO: 576 2804907 g292359 Human NFG genomic fragment. SEQ ID NO: 577 285202 g165652 protein kinase delta SEQ ID NO: 578 287240 g1009451 S.pombe chromosome I cosmid c22G7 SEQ ID NO: 579 287586 INCYTE INCYTE SEQ ID NO: 580 288492 g1291337 Soares fetal lung NbHL19W Homo sapiens cDNA clone 301455 5' similar to WP:W06B4.2 CE02891 SEQ ID NO: 581 289171 INCYTE INCYTE SEQ ID NO: 582 290214 INCYTE INCYTE SEQ ID NO: 583 290510 g1665778 Human mRNA for KIAA0256 gene, complete cds. SEQ ID NO: 584 290628 INCYTE INCYTE SEQ ID NO: 585 291736 INCYTE INCYTE SEQ ID NO: 586 2918759 g338630 Human synaptobrevin 2 (SYB2) gene, exon 5. SEQ ID NO: 587 2922560 INCYTE INCYTE SEQ ID NO: 588 029244 g756234 Homo sapiens cDNA clone 125197 5' similar to gb:M14565 CYTOCHROME P450 XIA1, MITOCHONDRIAL (HUMAN) SEQ ID NO: 589 292708 INCYTE INCYTE SEQ ID NO: 590 3038216 g1469884 KIAA0151 SEQ ID NO: 591 3043265 PubEST PubEST SEQ ID NO: 592 3044325 g788133 Homo sapiens cDNA clone 134940 5' similar to contains Alu repetitive element SEQ ID NO: 593 309389 INCYTE INCYTE SEQ ID NO: 594 3100562 g516680 Chicken gene for c-maf proto- oncogene product c-Maf, short form yv87e05 SEQ ID NO: 595 310202 g2415582 Homo sapiens mRNA for Marenostrin protein, complete. SEQ ID NO: 596 310487 g33942G Human T cell-specific protein (RANTES) mRNA, complete cds. SEQ ID NO: 597 3125445 g2224588 Human mRNA for KIAA0324 gene, partial cds. SEQ ID NO: 598 318358 g2224600 Human mRNA for KIAA0330 gene, partial cds. SEQ ID NO: 599 318438 INCYTE INCYTE SEQ ID NO: 600 318444 INCYTE INCYTE SEQ ID NO: 601 318774 g1064915 H.sapiens mRNA for ubiquitin conjugating enzyme, UbcH7. SEQ ID NO: 602 3188122 g2266637 Human OB-RGRP gene. SEQ ID NO: 603 3191066 g2280475 Human mRNA for KIAA0315 gene, partial cds. SEQ ID NO: 604 319684 INCYTE INCYTE SEQ ID NO: 605 320014 g1558796 Homo sapiens CDNA clone 525535 5' similar to SPA1 MOUSE P46062 GTPASE-ACTIVATING PROTEIN **SPA-1** SEQ ID NO: 606 320811 INCYTE INCYTE SEQ ID NO: 607 321651 INCYTE INCYTE SEQ ID NO: 608 334959 INCYTE INCYTE SEQ ID NO: 609 335100 INCYTE INCYTE SEQ ID NO: 610 336724 INCYTE INCYTE SEQ ID NO: 611 338196 INCYTE INCYTE SEQ ID NO: 612 338339 INCYTE INCYTE SEQ ID NO: 613 338345 INCYTE INCYTE SEQ ID NO: 614 338368 INCYTE INCYTE SEQ ID NO: 615 338435 INCYTE INCYTE SEQ ID NO: 616 339045 INCYTE INCYTE SEQ ID NO: 617 339198 INCYTE INCYTE SEQ ID NO: 618 339335 INCYTE INCYTE SEQ ID NO: 619 339678 INCYTE INCYTE SEQ ID NO: 620 339997 INCYTE INCYTE SEQ ID NO: 621 340100 INCYTE INCYTE SEQ ID NO: 622 340318 INCYTE INCYTE SEQ ID NO: 623 340422 INCYTE INCYTE SEQ ID NO: 624 340450 INCYTE INCYTE SEQ ID NO: 625 340883 INCYTE INCYTE SEQ ID NO: 626 341595 INCYTE INCYTE SEQ ID NO: 627 342342 g806765 Human 76 kDa tyrosine phosphoprotein SLP-76 mRNA, complete cds. SEQ ID NO: 628 343466 INCYTE INCYTE SEQ ID NO: 629 343595 g1184698 Human tyrosyl-tRNA synthetase mRNA, complete cds SEQ ID NO: 630 343619 BL00425A Arthropod defensins proteins. SEQ ID NO: 631 344012 INCYTE INCYTE SEQ ID NO: 632 345315 INCYTE INCYTE SEQ ID NO: 633 345380 g337810 Human MAR/SAR DNA binding protein (SATB1) mRNA, complete cds. SEQ ID NO: 634 345409 INCYTE INCYTE SEQ ID NO: 635 345472 INCYTE INCYTE SEQ ID NO: 636

346439 INCYTE INCYTE SEQ ID NO: 637 346597 g8651 Mst87F; structural sperm protein SEQ ID NO: 638 346869 g2257694 Homo sapiens mRNA for SCGF, complete cds. SEQ ID NO: 639 347184 g1197073 GEF1 SEQ ID NO: 640 003490 g30337 Human CYP2D7BP pseudogene for cytochrome SEQ ID NO: 641 349715 INCYTE INCYTE SEQ ID NO: 642 3518373 g2282039 Homo sapiens Arp2/3 protein complex subunit p20-Arc (ARC20) mRNA SEQ ID NO: 643 3523611 g1710211 Human clone 23732 mRNA, partial cds SEQ ID NO: 644 3534074 g19867 extensin (AA 1-620) SEQ ID NO: 645 3538629 g1386895 Soares fetal heart NbHH19W Homo sapiens cDNA clone 345320 5' simiiar to SWLCOGY_MOUSE Q02853 STROMELYSIN-3 PRECURSOR SEQ ID NO: 646 358673
g2465410 Homo sapiens Bcl-1/Bcl-2 binding protein (BAD) mRNA, partial cds SEQ ID NO: 647 361577 g189389 Homo sapiens osteogenic protein-2 (OP-2) mRNA, complete cds. SEQ ID NO: 648 369126 g1905905 Homo sapiens DNA from chromosome 19p13.2 cosmids R31240, R30272 and R28549 containing the EKLF, GCDH, CRTC, and RAD23A genes, genomic sequence. SEQ ID NO: 649 375230 g2505956 Rattus norvegicus mRNA for 70 kDa tumor specific antigen, partial.

Detailed Description Paragraph Table - DETL (9):

SEQ ID NO: 1084 g182860 Human glyceraldehyde-3-phosphate dehydrogenase mRNA, complete cds. SEQ ID NO: 1085 g182939 Human growth arrest and DNA-damage-inducible protein (gadd45) mRNA, SEQ ID NO: 1086 g183046 Human granulocyte colony-stimulating factor receptor (G-CSFR-1) mRNA, SEQ ID NO: 1087 g183083 Human basic fibroblast growth factor (bFGF) 22.5 kd, 21 kd and 18 kd SEQ ID NO: 1088 g183260 peroxidase (EC 1.11.1.9.). SEQ ID NO: 1089 g183361 Human GM-CSF receptor mRNA, complete cds. SEQ ID NO: 1090 g183390 Human granule membrane protein-140 mRNA, complete cds. SEQ ID NO: 1091 g183416 Human guanine nucleotide-binding protein G-s, alpha subunit mRNA, SEQ ID NO: 1092 g183442 H.sapiens zinc finger transcriptionai regulator mRNA, complete cds. SEQ ID NO: 1093 g183452 Human endothelial membrane glycoprotein IIIa (GPIIIa) mRNA, complete SEQ ID NO: 1094 g183484 Human Epstein-Barr virus induced G-protein coupled receptor mRNA, SEQ ID NO: 1095 g183497 cds, and platelet glycoprotein Ib beta chain SEQ ID NO: 1096 g183499 Human platelet glycoprotein Ib alpha chain mRNA, complete cds. SEQ ID NO: 1097 g183503 Human platelet glycoprotein IIb mRNA, 3' end. SEQ ID NO: 1098 g183612 H.sapiens granulin mRNA, complete cds. SEQ ID NO: 1099 g183628 Human cytokine (GRO-beta) mRNA, complete cds. SEQ ID NO: 1100 g183632 Human cytokine (GRO-gamma) mRNA, complete cds. SEQ ID NO: 1101 g183655 Human glutathione S-transferase mRNA, complete cds. SEQ ID NO: 1102 g18366i Homo sapiens glutathione transferase (GST) mRNA, with an 82 bp SEQ ID NO: 1103 g183666 Human glutathione S-transferase Ha subunit 2 (GST) mRNA, complete cds. SEQ ID NO: 1104 g183684 Human glucose transporter-like protein-III (GLUT3), complete cds. SEQ ID NO: 1105 g183911 Human hemopoietic cell protein-tyrosine kinase (HCK) gene, complete SEQ ID NO: 1106 g183915 Human hematopoietic cell phosphatase mRNA, complete cds. SEQ ID NO: 1107 g183944 Human sickle beta-hemoglobin mRNA. SEQ ID NO: 1108 g184081 Human histone H2A.1 (H2A) gene, complete cds. SEQ ID NO: 1109 g184227 Human 14 kDa beta-galactoside-binding lectin (114) gene, complete cds. SEQ ID NO: 1110 g184262 Human (clones 18, 23, 27, 24) c- myeloproliferative leukemia virus type SEQ ID NO: 1111 g184402 shock transcription factor 4, complete cds. SEQ ID NO: 1112 g184413 Human 70 kDa heat shock protein (hsp70) gene segment. SEQ ID NO: 1113 g184508 Human interleukin 9 receptor mRNA, complete cds. SEQ ID NO: 1114 g184524 Human integrin beta-5 subunit mRNA, complete cds SEQ ID NO: 1115 g184532 Human

major group rhinovirus receptor (HRV) mRNA, complete cds. SEQ ID NO: 1116 g184622 Human interferon-beta mRNA, complete cds. SEQ ID NO: 1117 g184636 (IFN-alpha-F) mRNA, complete cds. SEQ ID NO: 1118 g184645 Human interferon-alpha receptor (HuIFN-alpha- Rec) mRNA, complete cds. SEQ ID NO: 1119 g184650 Human interferon-gamma receptor mRNA, complete cds SEQ ID NO: 1120 g185361 Human (hybridoma H210) anti-hepatitis A IgG variable region, constant SEQ ID NO: 1121 g186264 Human gamma-interferon-inducible protein (IP-30) mRNA, complete cds. SEQ ID NO: 1122 g186270_2 mRNA, complete cds. SEQ ID NO: 1123 g186270_1 complete cds. SEQ ID NO: 1124 g186279 Homo sapiens interleukin 1 alpha (IL 1) mRNA, complete cds. SEQ ID NO: 1125 g186289 Human interleukin 1 receptor mRNA, complete cds. SEQ ID NO: 1126 g186322 Human interleukin 2 receptor beta chain (p70-75) mRNA, complete cds. SEQ ID NO: 1127 g186326 Human interleukin 3 (IL-3) mRNA, complete cds. SEQ ID NO: 1128 g186330 Human interleukin 3 receptor (hIL-3Ra) mRNA, complete cds. SEQ ID NO: 1129 g186334 Human interleukin 4 (IL-4) mRNA, complete cds. SEQ ID NO: 1130 g186346 (IL-6) receptor. SEQ ID NO: 1131 g186353 Human membrane glycoprotein gp130 mRNA, complete cds. SEQ ID NO: 1132 g186363 Human interleukin 7 (IL-7) mRNA, complete cds. SEQ ID NO: 1133 g186365 Human interleukin-7 receptor (IL-7) mRNA, complete cds. SEQ ID NO: 1134 g186369 Human IL-8 receptor mRNA, complete cds. SEQ ID NO: 1135 g186377 Human interleuken 8 receptor B mRNA, complete cds. SEQ ID NO: 1136 g186379 Human interleukin enhancer binding factor 2 (ILF2) mRNA. SEQ ID NO: 1137 g186439 Human insulin receptor mRNA, complete cds. SEQ ID NO: 1138 g186496 Human integrin alpha-3 chain mRNA, complete cds. SEQ ID NO: 1139 g186508 Human integrin beta-7 subunit mRNA, complete cds. SEQ ID NO: 1140 g186516 Human interleukin-8 receptor type B (IL8RB) mRNA, complete cds. SEQ ID NO: 1141 g186566 Homo sapiens interferon alpha induced transcriptional activator SEQ ID NO: 1142 g186567 Human transcription factor ISGF-3 mRNA sequence. SEQ ID NO: 1143 g186624 Human c-jun proto oncogene (JUN), complete cds, clone hCJ-1. SEQ ID NO: 1144 g186626 (JunB) gene, 5' region and complete SEQ ID NO: 1145 g186763 mRNA, complete cds. SEQ ID NO: 1146 g186933 Human leukocyte adhesion protein (LFA-1/Mac- 1/p150, 95 family) beta SEQ ID NO: 1147 g186935 Human CD11b (MAC-1/Mol/CR3) leukocyte adhesion receptor alpha subunit SEQ ID NO: 1148 g186965 Human lipopolysaccharide binding protein (LBP) mRNA, complete cds. SEQ ID NO: 1149 g186967 Human lipocortin-III mRNA, complete cds. SEQ ID NO: 1150 g187116 (leukocyte (neutrophil) elastase. SEQ ID NO: 1151 g187118 Human leukosialin mRNA, complete cds. SEQ ID NO: 1152 g187137 SEQ ID NO: 1153 g187172 Human leukotriene A-4 hydrolase mRNA, complete cds. SEQ ID NO: 1154 g187182 Human lymph node homing receptor mRNA, complete cds. SEQ ID NO: 1155 g187192 Human lipoxygenase mRNA, complete cds. SEQ ID NO: 1156 g187239 Human leukocyte surface protein (CD31) mRNA, complete cds. SEQ ID NO: 1157 g187262 Human B cell differentiation antigen mRNA, complete cds. SEQ ID NO: 1158 g187268 Human lyn mRNA encoding a tyrosine kinase. SEQ ID NO: 1159 g187273 Human eosinophil Charcot-Leyden crystal (CLC) protein SEQ ID NO: 1160 g187288 Homo sapiens antagonizer of myc transcriptional activity (Mad) mRNA, SEQ ID NO: 1161 g187290 Homo sapiens MAD-3 mRNA encoding IkB-like activity, complete cds. SEQ ID NO: 1162 g187386 Human myristoylated alanine-rich C-kinase substrate mRNA, complete SEQ ID NO: 1163 g187390 Human helix-loop-helix zipper protein (max) mRNA, complete cds. SEQ ID NO: 1164 g187414 Human eosinophil granule major basic protein mRNA, complete cds. SEQ ID NO: 1165 g187434 Human monocyte chemotactic and activating factor (MCAF) mRNA, complete SEQ ID NO: 1166 g187455 Homo sapiens macrophage capping protein mRNA, complete cds. SEQ ID NO: 1167 g187468 Human P-glycoprotein (MDR1) mRNA, complete cds. SEQ ID

NO: 1168 g187501 Human membrane glycoprotein P (mdr3) mRNA, complete cds. SEQ ID NO: 1169 g187701 Human MHC protein homologous to chicken B complex protein mRNA, SEQ ID NO: 1170 g188255 mRNA, complete cds. SEQ ID NO: 1171 g188469 Human major histocompatibility class II antigen gamma chain mRNA, SEQ ID NO: 1172 g188555 Human migration inhibitory factor (MIF) mRNA, complete cds. SEQ ID NO: 1173 g188568 Homo sapiens MAP kinase kinase mRNA, complete cds. SEQ ID NO: 1174 g188618 Human matrix metalloproteinase-3 (MMP-3) mRNA, complete cds. SEQ ID NO: 1175 g188623 Human monocyte activation antigen (Mo3) mRNA, complete cds. SEQ ID NO: 1176 g189050 Human 47-kD autosomal chronic granulomatous disease protein mRNA, SEQ ID NO: 1177 g189066 H.sapiens NAP (nucleosome assembly protein) mRNA, complete cds. SEQ ID NO: 1178 g189068 Human neutrophil adherence receptor alpha-M subunit mRNA. SEQ ID NO: 1179 g189105 Human neutrophil cytochrome b light chain p22 phagocyte b-cytochrome SEQ ID NO: 1180 g189177 Human nuclear factor kappa-B DNA binding subunit (**NF-kappa-B**) mRNA, SEQ ID NO: 1181 g189237 Human neuroleukin mRNA, complete cds. SEQ ID NO: 1182 g189243 Human N-myc oncogene protein mRNA. SEQ ID NO: 1183 g189267 Human neutrophil oxidase factor (p67-phox) mRNA, complete cds. SEQ ID NO: 1184 g189368 Human ornithine decarboxylase (ODC1) mRNA, complete cds. SEQ ID NO: 1185 g189501 Human 65-kilodalton phosphoprotein (p65) mRNA, complete cds. SEQ ID NO: 1186 g189537 Human platelet activating factor receptor mRNA, complete cds. SEQ ID NO: 1187 g189541 Human plasminogen activator inhibitor-1 (PAI-1) mRNA, complete cds. SEQ ID NO: 1188 g189544 Human placental plasminogen activator inhibitor mRNA, complete cds. SEQ ID NO: 1189 g189546 Human plasminogen activator inhibitor mRNA, complete cds. SEQ ID NO: 1190 g189616 Human protein PP4-X mRNA, complete cds. SEQ ID NO: 1191 g189679 Human protein kinase C-delta 13 mRNA, complete cds. SEQ ID NO: 1192 g189700 Human platelet-derived endothelial cell growth factor mRNA, complete SEQ ID NO: 1193 g189731 Human platelet-derived growth factor (PDGF) receptor mRNA, complete SEQ ID NO: 1194 g189733 Human platelet-derived growth factor receptor alpha (PDGFRA) mRNA, SEQ ID NO: 1195 g189846 Human perforin mRNA, complete cds. SEQ ID NO: 1196 g189940 Human phosphorylase kinase (PSK-C3) mRNA, complete cds. SEQ ID NO: 1197 g189946 Human homeobox protein (PHOX1) mRNA, 3' end. SEQ ID NO: 1198 g189995 Human pyruvate kinase type L mRNA, complete cds. SEQ ID NO: 1199 g190003 Human phosphatidylcholine 2-acylhydrolase (cPLA2) mRNA, complete cds. SEQ ID NO: 1200 g190012 Human lung phospholipase A-2 (PLA-2) mRNA, complete cds, clone SEQ ID NO: 1201 g190035 C. SEQ ID NO: 1202 g190039 Homo sapiens phospholipase C-beta-2 mRNA, complete cds. SEQ ID NO: 1203 g190339 Human perforin (PRF1) gene, complete cds. SEQ ID NO: 1204 g190419 Human secretory granule proteoglycan peptide core mRNA, complete cds. SEQ ID NO: 1205 g190734 Human protein-tyrosine kinase (JAK1) mRNA, complete cds. SEQ ID NO: 1206 g190827 Human rac protein kinase alpha mRNA, complete cds. SEQ ID NO: 1207 g190888 Human RASF-A PLA2 mRNA, complete cds. SEQ ID NO: 1208 g190899 Human (T3M-4) cellular transforming oncogene c- Ki-ras, exon 2. SEQ ID NO: 1209 g219534 Human CGM1a mRNA for CD66d. SEQ ID NO: 1210 g219587 Human mRNA for DnaJ protein homolog, complete cds. SEQ ID NO: 1211 g219667 Human plasma (extracellular) mRNA for glutathione peroxidase, complete SEQ ID NO: 1212 g219866 Human mRNA for HM74.

Detailed Description Paragraph Table - DETL (10):

SEQ ID NO: 1213 g219868 Human mRNA for HM89. SEQ ID NO: 1214 g219924 Human mRNA for MGC-24, complete cds. SEQ ID NO: 1215 g219928 Human midkine gene, complete cds. SEQ ID NO: 1216 g219935 Human CGM6 mRNA for CD66b (NCA-95). SEQ

ID NO: 1217 g220138 Human Pro-urokinase gene, complete cds. SEQ ID NO: 1218 g232582 HOX11 = HOX11 homeodomain [homeobox] [human, mRNA, 1988 nt] SEQ ID NO: 1219 g235648 tumor necrosis factor receptor = 75-kda [human, mRNA, 3492 nt] SEQ ID NO: 1220 g23896 Human placental cDNA coding for 5'nucleotidase (EC 3.1.3.5). SEQ ID NO: 1221 g243493 protein kinase inhibitor [human, neuroblastoma cell line SH-SY-5Y, SEQ ID NO: 1222 g243543 BPTP-1 = protein-tyrosine phosphatase [human, pre- B cell NALM-6, mRNA, SEQ ID NO: 1223 g243865 Ig gamma = immunoglobulin heavy chain [rats, humanized lympholytic MoAb SEQ ID NO: 1224 g243887 ubiquitin carboxyl extension protein [human, mRNA, 540 nt]. SEQ ID NO: 1225 g246741 CD8 beta 1 = T cell surface glycoprotein CD8 beta 1 chain [alternatively SEQ ID NO: 1226 g247306 cytochrome P450 reductase [human, placenta, mRNA Partial, 2403 nt]. SEQ ID NO: 1227 g250802 cathepsin S = cysteine proteinase [human, testis, mRNA, 1784 nt]. SEQ ID NO: 1228 g252001 GADD153 = growth arrest and DNA-damage-inducible gene [human SEQ ID NO: 1229 g258761 AMPD2 = AMP deaminase isoform L [human, T-lymphoblast and placenta, SEQ ID NO: 1230 g260573 transcription factor E2F like protein [human, mRNA, 2492 nt]. SEQ ID NO: 1231 g265702 A1-A2 lambda hybrid GAU heavy chain [membrane exon] [human, myeloma, mRNA Partial, 360 nt]. ACCESSION S55736 SEQ ID NO: 1232 g28343 H.sapiens ACTH-R gene for adrenocorticotropic hormone receptor. SEQ ID NO: 1233 g28711 H.sapiens encoding skin-derived antileukoproteinase. SEQ ID NO: 1234 g28805 Human mRNA for lipoprotein apoCII. SEQ ID NO: 1235 g28820 Human mRNA for A-raf-1 oncogene. SEQ ID NO: 1236 g288309 NO: 1236 g288309 H.sapiens mRNA for B cell differentiation factor I. SEQ ID NO: 1237 g28850 H.sapiens mRNA for arrestin (partial) SEQ ID NO: 1238 g28875 H.sapiens ash mRNA. SEQ ID NO: 1239 g28976 Human mRNA for azurocidin. SEQ ID NO: 1240 g291863 Human ADP-ribosylation factor-like mRNA (ARL2). SEQ ID NO: 1241 g291897 Homo sapiens early activation antigen CD69 mRNA, complete cds. SEQ ID NO: 1242 g291928 Homo sapiens Ig superfamily CTLA-4 mRNA, complete cds. SEQ ID NO: 1243 g292054 Human helix-loop-helix basic phosphoprotein (G058) mRNA, complete cds. SEQ ID NO: 1244 g292159 Human heat shock protein 70 (hsp70) mRNA, complete cds. SEQ ID NO: 1245 g292276 Homo sapiens lymphotoxin-beta mRNA, complete cds. SEQ ID NO: 1246 g292414 Homo sapiens zeta-crystallin/quinone reductase mRNA, complete cds. SEQ ID NO: 1247 g292416 Homo sapiens macrophage inflammatory protein-1- alpha/RANTES receptor SEQ ID NO: 1248 g292509 Human squalene synthetase (ERG9) mRNA, complete cds. SEQ ID NO: 1249 g29370 Human gene for beta-adrenergic receptor (beta-2 subtype) SEQ ID NO: 1250 g29388 H.sapiens mRNA for B-cell antigen CD75. SEQ ID NO: 1251 g29471 Human mRNA for B-myb gene. SEQ ID NO: 1252 g29537 Human mRNA for C1q B-chain of complement system. SEQ ID NO: 1253 g29645 H.sapiens mRNA for CAMPATH-1 (CDw52) antigen. SEQ ID NO: 1254 g29744 Human mRNA for Fc gamma receptor (FcRIII, CD16, FcR-1o). SEQ ID NO: 1255 g29773 Human mRNA for B lymphocyte antigen CD20 (B1, Bp35). SEQ ID NO: 1256 g29778 H.sapiens CD22 mRNA. SEQ ID NO: 1257 g297787 NO: 1257 g297787 H.sapiens interleukin-13 mRNA. SEQ ID NO: 1258 g29793 Human mRNA for leukocyte antigen CD37. SEQ ID NO: 1259 g29817 H.sapiens CD6 mRNA for T cell glycoprotein CD6. SEQ ID NO: 1260 g29819 Human mRNA for CD7 antigen (gp40). SEQ ID NO: 1261 g298303 interleukin-6 [human, tonsillar mononuclear cells, mRNA, 657 nt]. SEQ ID NO: 1262 g29850 Human CDw40 mRNA for nerve growth factor receptor-related B-lymphocyte SEQ ID NO: 1263 g298664 CD68 = 110 kda transmembrane glycoprotein [human, promonocyte cell line SEQ ID NO: 1264 g30125 H.sapiens mRNA for type I interstitial collagenase. SEQ ID NO: 1265 g30185 Human mRNA for complement receptor type 1 (CR1, C3b/C4b receptor, SEQ ID NO: 1266 g30220 Human mRNA for cripto protein. SEQ ID NO: 1267 g30255 Human

mRNA for C-SRC-kinase. SEQ ID NO: 1268 g30306 Human mRNA for cyclin A. SEQ ID NO: 1269 g30308 Human mRNA-for T-cell cyclophilin. SEQ ID NO: 1270 g303602 Human mRNA for cytochrome P-450LTBV. SEQ ID NO: 1270 g303602 Human mRNA for cytochrome P-450LTBV. SEQ ID NO: 1271 g303611 Human mRNA for interleukin 2 receptor gamma chain. SEQ ID NO: 1272 g306474 Homo sapiens tyrosine protein kinase (CAK) gene, complete cds. SEQ ID NO: 1273 g307184 Homosapiens ERK activator kinase (MEK2) mRNA. SEQ ID NO: 1274 g307299 Human **NF-kappa-B** transcription factor p65 subunit mRNA, complete cds. SEQ ID NO: 1275 g307387 Human ribosomal protein L7 (RPL7) mRNA, complete cds. SEQ ID NO: 1276 g307390 Human ribosomal protein S13 (RPS13) mRNA, complete cds. SEQ ID NO: 1277 g30820 H.sapiens mRNA for IFN-inducible gamma2 protein. SEQ ID NO: 1278 g31097 Human mRNA for elongation factor 1 alpha subunit (EF-1 alpha) SEQ ID NO: 1279 g311374 Human 1-8U gene from interferon-inducible gene family. SEQ ID NO: 1280 g311375 H.sapiens Humig mRNA. SEQ ID NO: 1281 g311699 H.sapiens GPx-4 mRNA for phospholipid hydroperoxide glutathione SEQ ID NO: 1282 g31192 H.sapiens granulin mRNA, complete cds. SEQ ID NO: 1283 g312141 H.sapiens mRNA for M130 antigen. SEQ ID NO: 1284 g312145 H.sapiens mRNA for M130 antigen cytoplasmic variant 2. SEQ ID NO: 1285 g312466 H.sapiens atk mRNA for agammaglobulinaemia tyrosine kinase. SEQ ID NO: 1286 g31296 H.sapiens FACC mRNA from complementation group C (FA(C)). SEQ ID NO: 1287 g31323 Human Fc-gamma RIII-2 cDNA for Fc-gamma receptor III-2 (CD16) SEQ ID NO: 1288 g31386 Human mRNA for fibroblast growth receptor 2-Ig domain. SEQ ID NO: 1289 g31396 Human mRNA for fibronectin (FN precursor). SEQ ID NO: 1290 g31421 Human mRNA for leukocyte-associated molecule-1 alpha subunit (LFA-1 SEQ ID NO: 1291 g31425 H.sapiens mRNA for five-lipoxygenase activating protein (FLAP). SEQ ID NO: 1292 g31437 Human mRNA for fibronectin receptor alpha subunit. SEQ ID NO: 1293 g31441 Human mRNA for fibronectin receptor beta subunit. SEQ ID NO: 1294 g31667 Human liver mRNA for beta-subunit signal transducing proteins Gs/Gi SEQ ID NO: 1295 g31880 H.sapiens mRNA for glutathione peroxidase-GI. SEQ ID NO: 1296 g32054 Human HS1 gene for heamatopoietic lineage cell specific protein. SEQ ID NO: 1297 g32432 Human mRNA for hematopoetic proteoglycan core protein. SEQ ID NO: 1298 g32449 Human mRNA encoding IMP:pyrophosphate phosphoribosyltransferase E.C. SEQ ID NO: 1299 g32455 H.sapiens hR-PTPu gene for protein tyrosine phosphatase. SEQ ID NO: 1300 g32477 Human mRNA for heat shock protein HSP27. SEQ ID NO: 1301 g32485 Human mRNA for heat shock protein hsp86. SEQ ID NO: 1302 g32691 Human mRNA for interferon IFN-gamma. SEQ ID NO: 1303 g32764 H. sapiens Ig constant region gene. SEQ ID NO: 1304 g32983 switch region (S epsilon). SEQ ID NO: 1305 g32998 Human DNA for insulin-like growth factor II (IGF-2); exon 7 and SEQ ID NO: 1306 g33058 Human mRNA for insulin-like growth factor I receptor. SEQ ID NO: 1307 g33780 Human mRNA encoding interleukin-2 (IL-2) a lymphozyte regulatory SEQ ID NO: 1308 g33789 Human mRNA for interleukin 1 beta. Peripheral blood mononuclear cells. SEQ ID NO: 1309 g338010 Human proteolytic serine esterase-like protein (SECT) gene, complete SEQ ID NO: 1310 g338020 Human sepiapterin reductase mRNA, complete cds. SEQ ID NO: 1311 g338079 Human tyrosine phosphatase mRNA, complete cds. SEQ ID NO: 1312 g33812 Human mRNA for interleukin-2 receptor. SEQ ID NO: 1313 g338227 Human src-like kinase (slk) mRNA, complete cds. SEQ ID NO: 1314 g33833 Human IL-4-R mRNA for the interleukin 4 receptor. SEQ ID NO: 1315 g33839 Human HSIL5R2 gene for interleukin-5 receptor type 2. SEQ ID NO: 1316 g338444 Human T-cell-specific homodimer surface protein CD28 mRNA, complete SEQ ID NO: 1317 g338633 Human syndecan mRNA, complete cds. SEQ ID NO: 1318 g33906 Human mRNA for integrin alpha-2 subunit. SEQ ID NO: 1319 g33910 Human mRNA for integrin

beta(4)subunit. SEQ ID NO: 1320 g33917 Human mRNA for gamma-interferon inducible early response gene (with SEQ ID NO: 1321 g339420 Human T cell-specific protein (RANTES) mRNA, complete cds. SEQ ID NO: 1322 g33945 Human mRNA for integrin alpha-4 subunit. SEQ ID NO: 1323 g33949 H.sapiens mRNA for integrin, alpha subunit. SEQ ID NO: 1324 g339515 Human transferrin receptor mRNA, complete cds. SEQ ID NO: 1325 g339569 Human TGF-beta type II receptor mRNA, complete cds. SEQ ID NO: 1326 g339656 Human endothelial cell thrombomodulin mRNA, complete cds. SEQ ID NO: 1327 g339706 Human metalloproteinase-2 inhibitor (TIMP-2) mRNA, complete cds. SEQ ID NO: 1328 g339708 Human thymidine kinase mRNA, complete cds. SEQ ID NO: 1329 g339737 Human tumor necrosis factor (TNF) mRNA. SEQ ID NO: 1330 g339744 Human tumor necrosis factor receptor mRNA, complete cds. SEQ ID NO: 1331 g339755 Human tumor necrosis factor receptor (TNF receptor) mRNA, complete SEQ ID NO: 1332 g339782 Human slow skeletal muscle troponin T mRNA, clone M1. SEQ ID NO: 1333 g340306 Human cell adhesion protein (vitronectin) receptor alpha subunit mRNA, SEQ ID NO: 1334 g340396 complete cds. SEQ ID NO: 1335 g34084 Human c-kit proto-oncogene mRNA. SEQ ID NO: 1336 g34266 Human mRNA for LCA-homolog. LAR protein (leukocyte antigen related). SEQ ID NO: 1337 g34275 Human mRNA for T200 leukocyte common antigen (CD45, LC-A) SEQ ID NO: 1338 g34280 Human mRNA for leukocyte common antigen (T200). SEQ ID NO: 1339 g34288 Human mRNA for lck tyrosine kinase. SEQ ID NO: 1340 g34312 Human mRNA for lactate dehydrogenase-A (LDH-A, EC 1.1.1.27) SEQ ID NO: 1341 g34346 Human mRNA for lymphocyte function associated antigen-3 (LFA-3). SEQ ID NO: 1342 g34387 Human mRNA for lipocortin. SEQ ID NO: 1343 g34444 Human mRNA for lymphotoxin.

US-PAT-NO: 6569624

DOCUMENT-IDENTIFIER: US 6569624 B1

TITLE: Identification of genetic markers of biological age and metabolism

DATE-ISSUED: May 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Prolla; Tomas A.	Madison	WI	N/A	N/A
Lee; Cheol-Koo	Madison	WI	N/A	N/A

APPL-NO: 09/ 630567

DATE FILED: August 8, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to provisional application No. 60/148,540, filed Aug. 12, 1999, U.S. provisional application No. 60/178,232, filed Jan. 26, 2000 and U.S. provisional application No. 60/211,923 filed Jun. 16, 2000. These provisional applications are incorporated by reference as if fully set forth herein.

US-CL-CURRENT: 435/6, 536/24.3

ABSTRACT:

A method of measuring the biological age of a multicellular organism is disclosed. In one embodiment this method comprises the steps of obtaining a sample of nucleic acid isolated from the organism's organ, tissue or cell and determining the expression pattern of a panel of sequences within the nucleic acid that have been predetermined by either increase or decrease in response to biological aging of the organ, tissue or cell. A method of obtaining biomarkers of aging is also disclosed. This method comprises the step of comparing a gene expression profile of a young multicellular organism subject's organ, tissue or cells; a gene expression profile from a chronologically aged subject's organ, tissue or cell; and a gene expression profile from a chronologically aged but biologically younger subject's organ, tissue or cell and identifying gene expression alterations that are observed when comparing the young subjects and the chronologically aged subjects and are not observed or reduced in magnitude when comparing the young subjects and the chronologically aged but biologically younger subjects.

7 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Detailed Description Paragraph Table - DETL (8):

TABLE 8 Caloric restriction-related decreases in gene expression in neocortex ot C57BL/6 mice* CR Signal Intensity ORF Increase SE CR Control Gene Class X76505 -7.2 1.0 -195 73 Tyro 10 Signal transduction U43088 -6.3 1.1 -109 164 IL-17 (CTLA-8) Immune/inflammatory W50186 -5.6 2.1 -38 129 Heavy chain homolog Unknown Y07711 -3.5 0.5 28 151 Zyxin Signal transduction Z47205 -3.1 0.8 45 200 PLZF Transcriptional factor AA000203 -2.8 0.7 -93 26 Corticosteroid-binding globulin precursor Transport W83658 -2.6 0.5 51 197 Guanine nucleotide-binding protein Signal transduction G(I)/G(S)/G(O) homolog L46815 -2.6 0.2 8 67 Ig kappa chain recombination and transcription DNA metabolism enhancer AA153484 -2.4 0.5 208 456 SERCA2 Ion transport W51466 -2.4 0.4 12 147 Chlorine channel protein P64 homolog Unknown U27398 -2.4 0.4 39 132 XPC DNA Metabolism X58069 -2.2 0.7 54 164 H2A.X DNA metabolism U50712 -2.2 0.4 54 156 MCP-5 Immune/inflammatory M61909 -2.1 0.3 39 125 **NF-kappa-B** p65 Stress response AA072643 -2.1 0.4 49 110 Midkine precursor homolog Stress response L01991 -2.1 0.3 48 132 PANG Unknown L04678 -2.1 0.2 -64 138 Integrin beta 4 subunit Structural W64628 -2.1 0.4 62 197 Guanine nucleotide-binding protein Signal transduction G(I)/G(S)/G(O) gamma-7 subunit X54098 -2.0 0.3 55 136 lamin B2 Structural AA023458 -2.0 0.3 20 107 Heat shock 27 KD protein homolog Stress response D63380 -2.0 0.2 -19 32 Alpha-1,3-fucosyltransferase Protein metabolism U15548 -2.0 0.3 -30 42 Beta 2 thyroid hormone receptor Energey metabolism AA123385 -2.0 0.2 57 117 Phosphorylase B kinase gamma catalytic chain Energey metabolism X57349 -2.0 0.4 -10 49 Transferrin receptor Transport D00659 -2.0 0.1 1 35 Aromatase P450 Biosynthesis AA028875 -2.0 0.2 -32 54 Glycine-rich cell wall structural homolog Lysosomal X76291 -2.0 0.1 11 79 Ihh (Indian Hedgehog) Signal transduction AA041982 -1.9 0.3 44 84 LARK Circadian regulation AA118758 -1.9 0.2 103 206 Multifunctional aminoacyl-tRNA-synthetase Protein synthesis W75353 -1.9 0.3 90 162 Apolipoprotein C-IV Transport W55410 -1.9 0.2 30 111 Tubulin gamma chain homolog Unknown L20343 -1.9 0.2 22 102 L-type calcium channel beta 2a subunit isoform Transport W91095 -1.9 0.5 44 93 Valyl-tRNA synthetase Protein metabolism X81593 -1.9 0.1 53 119 Winged-helix domain Transcriptional factor M38248 -1.9 0.2 -6 25 BALB8N Unknown J04694 -1.9 0.3 48 134 Alpha-1 type IV collagen Structural L47650 -1.8 0.3 50 85 STAT6 R Immune/inflammatory AA023595 -1.8 0.1 38 133 Frizzled protein precursor Signal transduction AA015168 -1.8 0.2 42 97 Interferon-gamma receptor beta chain homolog Immune/inflammatory AA013951 -1.8 0.1 32 38 Creatine transporter homolog Energey metabolism W78443 -1.8 0.2 17 106 MKP-X Signal transduction D31842 -1.8 0.2 66 126 PTP36 Structural W50138 -1.8 0.2 1 162 Putative serine/threonine-protein kinase B0464.5 Unknown L35307 -1.8 0.2 33 104 c-Knox Transcriptional factor AA073154 -1.8 0.3 31 68 Alpha-catenin homolog Structural W12720 -1.8 0.3 149 251 RAP-2B homolog Signal transduction AA170169 -1.8 0.2 -17 37 Elongation factor 1-gamma homolog Protein metabolism W48951 -1.8 0.3 8 30 Voltage-dependent anion-selective channel Unknown protein 2 homolog M35732 -1.8 0.3 -13 17 Seminal vesicle secretory protein IV Unknown AA145515 -1.8 0.3 68 187 Pre-MRNA splicing factor PRP6 RNA metabolism

W13162 -1.8 0.1 -7 62 Cell division protein kinase 4 DNA metabolism J03482 -1.8 0.2 42 113 Histone H1 DNA metabolism W82793 -1.8 0.1 -4 59 Topoisomerase E III homolog DNA metabolism Z31360 -1.8 0.3 1 51 P/L01 Unknown Y09632 -1.8 0.1 16 37 Rabkinesin-6 Transport AA066621 -1.8 0.2 13 63 60S ribosomal protein L10 Protein metabolism U67874 -1.8 0.3 46 85 Ubiquitin thiolesterase family Protein metabolism AA109714 -1.8 0.3 562 968 SKP1 RNA metabolism AA007957 -1.8 0.2 210 357 Threonyl-tRNA synthetase homolog Protein metabolism AA162633 -1.8 0.2 46 95 Isoleucyl-tRNA synthetase Protein metabolism M17299 -1.8 0.3 29 101 Phosphoglycerate kinase (pgk-2) Energy metabolism AA050102 -1.7 0.3 211 263 Elongation factor 2 (EF-2) Protein metabolism W54637 -1.7 0.2 72 37 Tubulin beta-2 chain class-II homolog Unknown D10028 -1.7 0.3 167 312 Glutamate receptor channel subunit zeta 1 Neurotransmission M28587 -1.7 0.2 -52 30 Alpha leukocyte interferon Immune/inflammatory AA023506 -1.7 0.2 60 144 Insulin receptor substrate-3 Energey metabolism W70629 -1.7 0.3 92 158 COP-II Protein metabolism U33626 -1.7 0.3 66 125 PML isoform 1 (Pml) Unknown AA144746 -1.7 0.2 42 92 EF-1-delta Protein metabolism M19380 -1.7 0.3 1406 2303 Calmodulin (Cam III) Signal transduction AA144136 -1.7 0.2 43 100 Choline kinase Rt homolog Biosynthesis AA165847 -1.7 0.3 331 509 EF-1-alpha2 homolog Protein metabolism W33415 -1.7 0.2 90 136 ATP citrate-lyase Unknown U35233 -1.6 0.1 71 109 Endothelin-1 Vasoconstrictive peptide W57384 -1.9 0.3 6 15 ATP synthase A chain homolog Energey metabolism X60452 -1.6 0.3 124 200 Cytochrome P-450IIIA Stress response AA02227 -1.6 0.1 172 279 Vascular endothlial growth factor Unknown AA168841 -1.6 0.2 169 289 Serine/threonine-protein kinase PAK Unknown AA120586 -1.6 0.1 9 64 Apolipoprotein B-100 precursor Stress response AA104561 -1.6 0.2 104 166 EIF-4A homolog Protein metabolism X17071 -1.6 0.1 25 90 Trophoblast-specific protein Growth factor M96265 -1.6 0.1 153 250 Galactose-1-phosphate uridyl transferase Biosynthesis AA145160 -1.6 0.2 178 287 Translational initiation factor 2 alpha Protein metabolism X63473 -1.6 0.1 69 110 m4 muscannic acetylcholine receptor Neurotransmission AA002750 -1.5 0.2 176 290 5-lipoxygenase activating protein (FLAP) Immune/inflammatory W64698 -1.5 0.2 51 63 Protein kinase C inhibitor 1 Signal transduction U63841 -1.5 0.1 120 197 NeuroD3 Growth factors U04294 -1.5 0.1 99 150 Potassium channel subunit (m-eag) Transport M33227 -1.5 0.2 259 396 Cryptdin-related (CRS4C) Immune/inflammatory U20532 -1.5 0.1 45 67 P45 NF-E2 related factor 2 (Nrf2) Transcriptional factor AA140026 -1.5 0.1 378 519 DNA directed RNA polymerase polypeptide G DNA metabolism W09025 -1.5 0.1 47 68 ATP synthase B chain homolog Energey metabolism W29163 -1.5 0.1 342 465 Leydig cell tumor 10kd protein homolog Unknown AA155191 -1.5 0.1 36 65 Kinesin heavy chain Transport M80363 -1.5 0.1 63 96 Rep-3 DNA metabolism AA044561 -1.4 0.2 93 132 PEP carboxykinase - mitochondrial Energey metabolism AA096843 -1.4 0.2 130 175 Unknown Unknown X57277 -1.4 0.1 908 1298 Rac 1 Signal transduction W82998 -1.4 0.1 256 363 BUB3 DNA metabolism *The values presented for Signal Intensity are the averages of three mice per age group and are expressed as data for old CR/old control mice. The SE was calculated for the nine pairwise comparisons and was obtained by dividing the standard deviation by the square root of 3. The method from which signal intensity is used to estimate fold changes is described in the Methods section of the manuscript.

Detailed Description Paragraph Table - DETL (9):

TABLE 9 Aging-related increases in gene expression in the cerebellum of C57B/6 mice* Fold Signal Intensity CR ORF Change SE Old Young Gene Class

Prevention AA120109 9.3 3.4 254 29 Interferon-induced protein 6-16 precursor Immune/inflammatory N M21050 6.4 0.9 291 14 Lysozyme P (Lzp-s) Immune 88 X56824 5.7 1.9 160 89 Tumor-induced 32 kD protein (p32) Unknown 100 V00727 5.6 2.6 282 57 c-fos Stress 30 M13019 4.9 0.7 109 3 Thymidylate synthase DNA metabolism 87 L16894 4.7 1.0 192 5 Cyclophilin C (CyCAP) Immune/inflammatory N AA146437 4.7 0.3 841 169 Cathepsin S precursor Stress 62 X58861 4.4 0.2 719 160 C1Q alpha-chain Immune/inflammatory 80 W67046 4.3 0.8 50 1 C6 chemokine Immune/inflammatory N X66295 4.1 0.6 508 147 C1q C-chain Immune/inflammatory 56 W65899 4.1 1.8 152 58 Guanine nucleotide-binding protein Signal transduction 80 U00677 4.1 2.2 16 -10 Syntrophin-1 Neuratransmission 100 X68273 3.9 1.8 108 -37 Macrosialin Immune/inflammatory N U19854 3.9 0.5 35 -63 Ubiquitinating enzyme E2-20K Protein metabolism 100 U63133 3.9 1.1 318 95 Emv-3 Viral N L20315 3.8 0.1 97 26 MPS1 Immune/inflammatory 56 K01347 3.8 0.7 337 109 Glial fibrillary acidic protein (GFAP) Stress 61 M17440 3.7 0.3 445 116 Sex-limited protein (SlpA) Immune/inflammatory N X91144 3.6 1.3 38 -2 P-selectin glycopratetn ligand 1 Immune/inflammatory 100 U43084 3.5 0.8 54 18 IFIT-2 Glucocorticoid-attenuated response Immune/inflammatory N AA089333 3.4 0.2 208 61 Cathepsin S precursor Stress 71 X83733 3.4 0.3 71 -7 SAP62-AMH RNA metabolism 100 W45750 3.3 1.3 197 257 Guanine nucleotide-binding protein G(T) Signal transduction 100 M22531 3.3 0.2 431 146 Clq B-chain Immune/inflammatory 65 AA031244 3.1 0.4 83 9 DNAJ protein homolog HSJ1 Stress 100 M60429 3.1 0.8 121 37 Ig-gamna 1 chain Immune/inflammatory 100 AA036067 3.0 0.4 815 311 Apolipoprotein E precursor (APO-E) Lipid transport 28 U06119 2.9 0.3 27 4 Cathepsin H prepropeptide (ctsH) Stress response 55 AA106347 2.9 0.3 243 57 Angiotensinogen precursor Osmoregulation 80 W98998 2.9 0.7 182 79 Neurogenic locus notch homolog protein 1 Immune/inflammatory 100 AA059700 2.8 0.3 2013 687 MHC class 1 B(2)-microglobulin Immune/inflammatory 45 U73037 2.8 0.8 69 41 Interferon regulatory factor 7 (mirf7) Immune/inflammatory 50 Y00964 2.8 0.3 780 316 beta-hexosaminidase (Hexb) Unknown 47 X55315 2.8 0.6 63 15 Fetus cerebral cortex for 3UTR Transcription factor 100 U37465 2.8 0.1 15 -7 Protein tyrosine phosphatase phi (PTPphi) Unknown 63 L07803 2.7 1.2 24 -15 trombospondin 2 Structural N U19119 2.7 0.3 52 -5 G-proten-like LRG-47 Immune/inflammatory N X52886 2.6 0.2 893 326 Cathepsin D Stress response 38 W70578 2.6 1.2 31 7 Antigen WC1.1 Immune/inflammatory 81 X16705 2.6 0.4 93 -4 Laminin B1 Structural 84 W57539 2.6 0.3 28 6 Oocyte zinc finger protein XLCOF8 Unknown N X52308 2.6 0.4 32 9 Thrombin Fibrinogen activation 91 U70859 2.6 0.7 109 46 Cationic amino acid transporter (CAT3) AA transport 49 U41497 2.6 1.1 160 40 Very-long chain acyl-CoA dehydrogenase Lipid metabolism 100 AA089339 2.6 0.5 76 31 Cystatin C precursor Immune/inflammatory 100 X16151 2.5 0.1 239 95 Early T-lymphocyte activation 1 protein Immune/inflammatory 49 U37419 2.5 0.5 111 -2 G protein alpha subunit (GNA-15) Unknown N K02785 2.5 0.5 15 -6 r-tos Stress response N M12289 2.5 0.5 39 25 Pennatal sketetal myosin heavy chain Structural 100 X58849 2.4 0.4 59 13 Murine Hox-4.7 Developmental 100 AA063858 2.4 0.2 89 32 Rho-related GTP-binding protein RHOG Signal transduction 74 D10632 2.4 0.2 33 -27 Zinc finger protein Transcription factor N U33005 2.3 0.4 35 -8 tbc 1 Unknown N W85160 2.3 0.7 70 41 40S ribosomal protein S4.X isoform Unknown 100 U57331 2.3 1.0 42 15 Transcription factor Tbx6 (tbx6) Developmental 92 U44731 2.3 0.2 71 20 Putative purine nucleotide binding protein Immune/inflammatory N W87253 2.3 0.6 58 16 Integrin beta-5 subunit precursor Cell adhesion 100 U53142 2.3 0.2 223 101 Endothelial constitutive nitric oxide syntnase Neurotransmission N AA087715 2.3 0.1 85 -61 GTPase-activating protein **SPA-1**

Unknown N D49429 2.3 0.3 554 251 Rad21 homolog DNA metabolism 73 AA155318 2.3 0.4 291 129 HNRP1 RNA metabolism N AA032593 2.3 0.1 99 17 Transducin beta chain 2 Signal transduction 83 X03690 2.3 0.2 45 -13 Ig mu chain Immune/inflammatory 93 M26417 2.3 0.5 54 28 T cell receptor beta chain Immune/inflammatory 100 X86374 2.2 0.6 73 38 TAG7 Immune/inflammatory 38 W90894 2.1 0.3 27 -11 Cell division protein kinase 4 DNA metabolism 100 M84005 2.2 0.7 83 51 Olfactory receptor 15 Odor receptor 23 X55573 2.2 0.5 55 19 Brain-derived neurotrophic factor Growth factor N W30129 2.2 0.3 90 -16 Phosphatidylinositol glycan homolog Structural 100 AA163771 2.2 0.3 153 67 EIF-2B epsilon subunit Protein metabolism N X72910 2.1 0.4 96 44 HSA-C Unknown N AA116604 2.1 0.2 303 181 Cathepsin Z Stress response 64 L16462 2.1 0.4 51 4 BCL2-related protein A1 Apoptosis 58 L13732 2.1 0.4 53 29 Natl. resistanc-asstd. macrophage protein 1 Immune/inflammatory 85 D37791 2.1 0.1 934 424 Beta-1,4-galactosyltransferase Protein metabolism 82 AA125097 2.0 0.1 618 313 Unknown Unknown 94 AA109998 2.0 0.2 40 12 Hexokinase D homolog Energy metabolism 100 M88127 2.0 0.2 33 -8 APC2 homolog Unknown 82 X13538 2.0 0.5 114 45 Hox-1,4 Growth/development 100 V01527 2.0 0.5 28 10 H2-IA-beta Immune/inflammatory 100 AA144411 2.0 0.1 86 79 Unknown Unknown 100 X63535 2.0 0.1 55 21 Tyrosine-protein kinase receptor UFO Signal transduction N M83348 2.0 0.1 42 22 Pregnancy specific glycoprotein homolog Unknown N W08211 2.0 0.2 62 26 TGF-beta receptor type III Signal transdtuction 100 W13136 2.0 0.4 266 87 Angiotenisinogen Osmoregulation 36 W46084 2.0 0.1 89 45 Unknown Unknown N U73744 2.0 0.1 3958 2909 Heat shock 70 Stress response 100 D29763 1.9 0.2 465 271 Seizure-related, product 6 type 3 Unknown 47 AA118121 1.9 1.0 51 37 Isoleucyl-IRNA synthetase Protein metabolism N M27034 1.9 0.2 258 163 MHC ctass 1 D-region Immune/inflammatory N U35249 1.9 0.1 68 36 CDK-activating kinase assembly factor DNA metabolism 61 J03776 1.9 0.4 37 22 Down regulatory protein (rpt-1r) of IL-2 Immune/inflammatory N receptor U28728 1.9 0.3 221 112 Els Signal transduction 66 AA124192 1.9 0.2 411 244 Unknown Unknown 44 W63809 1.8 0.4 136 80 Unknown Unknown 73 X16834 1.8 0.2 455 182 Galectin-3 Immune/inflammatory N X16995 1.8 0.2 351 221 N10 nuclear hormonal receptor homolog Unknown 100 J02870 1.8 0.2 848 380 40S ribosomal protein SA Protein metabolism 100 L21768 1.8 0.2 153 76 EGF 15 Growth factor 68 AA117284 1.8 0.1 217 123 Zinc finger protein homolog Unknown N *The values presented for Signal Intensity are the averages of three mice per age group and are expressed as data for old/young mice. The prevention by CR is shown as being none (N) or the calculated percentage effect. The SE was calculated for the nine pairwise comparisons and was obtained by dividing the standard deviation by the square root of 3. The method which signal intensity is used to estimate fold changes is described in the Methods section of the manuscript.

Detailed Description Paragraph Table - DETL (10):

TABLE 10 Aging-related decreases in gene expression in the cerebellum of C57B/6 mice* Fold Signal Intensity CR ORF Change SE Old Young Gene Class Prevention U00445 -4.3 1.4 39 132 Glucose-6-phosphatase Energy metabolism 79 W48504 -4.1 1.1 32 78 phosphoneuroprotein 14 homolog) Unknown N AA153337 -3.9 0.7 67 218 Myosin regulatory light chain 2 (MLC-2) Unknown 61 W51213 -3.9 0.5 14 57 NEDD-4 homolog Protein metabolism 55 X56304 -3.1 0.4 2 27 Tenascin Growth/development N W12681 -3.1 0.6 30 126 Hepatocyte growth factor Growth/development 37 Z68889 -2.9 1.0 30 70 Wnt-2 homolog Growth/development N W55684 -2.8 0.6 13 37 Brain protein i47 Unknown N U04827 -2.8 0.5 94 219

Brain fatty acid-binding protein (B-FABP) Growth/development N AA008066 -2.7 1.0 1 61 Pre-mRNA splicing factor PRP22 Unknown 74 W55300 -2.7 0.7 20 47 Fatty acid-binding protein, heart (H-FABP) Unknown 71 D13903 -2.7 0.5 7 37 MPTPdelta (type A) Growth/development N AA013976 -2.6 0.5 162 405 POL polyprotein; reverse transcriptase Unknown N ribonuclease H W10865 -2.6 0.2 14 142 Myosin light chair 1, atrial/foetal isoform Unknown N AA020296 -2.5 0.2 -162 166 NG9 Growth/development 100 W64865 -2.5 1.1 10 31 Stat-3 Unknown N AA139694 -2.5 0.3 64 203 Beta-myosin heavy chain Transport 100 U29762 -2.5 0.3 304 657 Albumin gene D-Box binding protein Transcription Factor N M87276 -2.4 0.5 16 34 Thrombospondin Structural 52 X02677 -2.4 0.2 63 160 Anion exchange protein Anion exchanger 100 X04836 -2.4 0.2 22 68 T-cell antigen CDA Immune/inflammatory 100 X87242 -2.4 0.3 48 111 unc-33 Growth/development 70 AA163021 -2.4 0.2 28 43 Annexin VIII Signal transduction 84 M31810 -2.4 0.3 29 113 P-protein membrane transporter Transport 100 M97900 -2.4 0.6 18 49 Unknown Unknown 20 M15008 -2.4 0.6 101 227 Steroid 21-hydroxylase B Steroid metabolism 100 M99377 -2.4 0.5 77 191 Alpha-2 adrenergic receptor Neurotransmission N M32490 -2.4 0.3 62 122 Cyr61 Growth/development 41 AA168350 -2.3 0.3 130 237 Cysteinyl-tRNA synthetase Protein metabolism 83 AA061206 -2.3 0.2 8 52 Unp (ubiquitin protease) Protein metabolism N W12794 -2.3 0.3 23 96 Unknown Unknown 78 AA050593 -2.3 0.1 5 69 Unknown Unknown 62 AA050715 -2.3 0.3 64 148 Smoothelin Structural 92 AA106463 -2.2 0.3 110 277 Phosphoenolpyruvate carboxykinase Energy metabolism N X90829 -2.2 0.3 -16 9 Lbx1 Growth/development N X65588 -2.2 0.3 -1 24 mp41 Neurotransmission N J00475 -2.2 0.2 -23 58 Ig alpha chain Immune/inflammatory N X03019 -2.2 0.3 4 71 GM-CSF Immune/inflammatory 26 W34687 -2.2 0.4 62 115 Alpha-actin Transport 78 W75614 -2.2 0.4 27 56 Alpha-synuclein Growth/development N AA068153 -2.2 0.3 14 39 Polyadenylate-binding protein RNA metabolism 55 U36842 -2.1 0.5 22 36 Riap 3-inhibitor of apoptosis Apoptosis 100 W09127 -2.1 0.3 3 85 60S ribosomal protein L22 Protein metabolism 100 D63819 -2.1 0.2 29 87 Neuropeptide Y-Y1 receptor Neurotransmission N M33884 -2.1 0.1 70 139 Env polyprotein Viral protein 55 AA144430 -2.1 0.3 64 156 **NF-KB** P100 inhibition subunit Stress response 48 AA168554 -2.1 0.3 119 246 Unknown Unknown 85 U35730 -2.1 0.8 12 30 Jerky Unknown N M92649 -2.1 0.4 45 112 nitric oxide syntnase Neurotransmission N D12907 -2.1 0.2 55 126 Serine protease inhibitor homologue Unknown 85 M17327 -2.1 0.2 234 566 Env polyprotein Viral protein 56 AA170444 -2.1 0.2 172 246 Ubiquitin-activating enzyme E1 Protein metabolism 100 W12658 -2.1 0.3 203 415 FKBP-rapamycin associated protein Unknown N AA123026 -2.1 0.3 60 116 REG 2 Unknown 100 W13125 -2.1 0.5 111 232 Phenylalanyl-tRNA synthetase beta chain Protein metabolism N AA103862 -2.1 0.4 53 143 Unknown Unknown N U21301 -2.1 0.6 30 62 c-mer tyrosine kinase receptor Signal transduction N W13586 -2.1 0.1 29 136 Myosin light chain 1 homolog Transport 100 W42217 -2.1 0.1 69 143 Ribosonal protein S20 Protein metabolism 100 AA153522 -2.1 0.4 95 191 Serine/threonine kinase Signal transducticn 78 W30612 -2.0 0.1 70 160 Chloride intracellular channel 3 Transport 100 W11621 -2.0 0.4 78 138 Zinc finger protein 126 Unknown N X72805 -2.0 0.3 25 63 CD-1 histone H1t DNA metabolism N L08407 -2.0 0.3 38 117 Collagen type XVII Structural N AA145609 -2.0 0.2 55 134 cAMP responsive element modifier Transcripional factor 34 W12756 -2.0 0.1 48 117 Unknown Unknown 92 W75523 -2.0 0.3 48 95 Vertebrate homolog of C. elegans Lin-7 Unknown N type 2 D85904 -1.9 0.3 69 129 Heat shock 70-related protein Apg-2 Stress response N AA138911 -1.8 0.2 176 311 RNA helicase PRP16 RNA metabolism 100 W42216 -1.8 0.1 183 361 SWI/SNF related homolog Transcriptional factor 74 W12395 -1.8 0.4 141 237 Transcription elongation factor A (SII)

Transcriptional factor 88 K03235 -1.8 0.1 84 149 Proliferin 2 Growth factor 100 AA145859 -1.8 0.1 4110 5250 Unknown Unknown 100 W57194 -1.8 0.2 61 108 Ubiquitin carboxyl terminal hydrolase 12 Protein metabolism N AA166440 -1.7 0.1 229 389 Phosphatidylserine decarboxylase Protein metabolism N L33726 -1.7 0.1 69 128 Fascin homolog 1 Structural 100 L35549 -1.7 0.4 30 38 Y-box binding protein homolog Unknown 100 AA154514 -1.7 0.1 7639 12878 ATP synthase A chain (protein 6) homolog Energy metabolism 100 AA143937 -1.7 0.1 384 697 Beta-centractin Transport 70 AA027387 -1.7 0.1 169 270 Rab-4B Transport 51 L38971 -1.7 0.2 205 334 Integral membrane protein 2 Unknown 43 W10526 -1.7 0.1 193 301 Ca.sup.- channel, voltage-dep., gamma subunit 1 Transport 90 W12204 -1.6 0.2 114 200 Ca2+/calmodulin-dependent protein kinase Signal transduction N isoform gamma B AA170173 -1.6 0.1 149 289 NTT-73 Transport 100 M64403 -1.6 0.1 126 208 Cyclin D1 homolog DNA metabolism 100 W13191 -1.6 0.1 288 347 Thyroid hormone receptor alpha 2 Energy metabolism 87 U47543 -1.6 0.1 121 205 NGF1-A binding protein 2 (NAB2) Growth factor N D70848 -1.6 0.2 154 246 Zic2 (cerebellar zinc finger protein) Neural development 77 X56518 -1.6 0.3 106 164 Acetylcholinesterase Neurotransmission N AA144588 -1.6 0.2 233 368 Beta-adrenergic receptor kinase 2 homolog Neurotransmission 33 AA139828 -1.6 0.1 224 351 gonadotropin inducible transcription Unknown 100 repressor-1 homolog AA061170 -1.6 0.2 43 65 WW-domain oxidoreductase homolog Unknown N X58287 -1.6 0.3 84 153 mR-PTPu Signal transduction N L13129 -1.6 0.1 162 220 Annexin A7 Exocytossis 90 D85037 -1.6 0.1 50 77 Doc2beta Neruotransmission N U30823 -1.6 0.2 55 102 Myocyte enhancer factor-2A Transciptional factor 33 W64791 -1.6 0.1 92 143 Galactokinese Energy metabolism N X52622 -1.6 0.1 274 377 IN Viral protein 100 AA063914 -1.5 0.1 175 267 Alpha-tubulin Transport 64 *The values presented for Signal Intensity are the averages of three mice per age group and are expressed as data for old/young mice. The prevention by CR is shown as being none (N) or the calculated percentage effect. The SE was calculated for the nine pairwise comparisons and was obtained by dividing the standard deviation by the square root of 3. The method from which siganl intensity is used to estimate fold changes is described in the Methods section of the manuscript.